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Epidemiology and Management of High Pathogenicity Avian Influenza in South African Coastal Seabirds, 2018-2022

by

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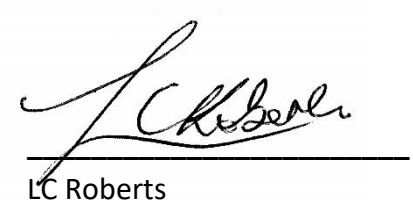
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Declaration

I, Laura Christl Roberts (student number 26217369) hereby declare that the dissertation which I hereby submit for the degree PhD in the Faculty of Veterinary Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.



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Abstract

High pathogenicity avian influenza (HPAI) is a viral disease of birds that has caused increasing harm to global poultry production and food security and, more recently, to the conservation of wild bird and marine mammal populations. Avian influenza viruses are also closely monitored because of their inherent human pandemic potential. Since 2016, Clade 2.3.4.4b viruses of the Goose-Guangdong HPAI lineage have spread from Asia to all continents, except Oceania, in an unprecedented manner. Two different clade 2.3.4.4b viruses reached South Africa in 2018 and 2021 respectively and at least thirty thousand South African coastal seabirds died, mostly Endangered Cape Cormorants and African Penguins. This was followed by severe seabird outbreaks elsewhere around the globe and increasing discussion around suitable response measures, which have remained limited.

This dissertation aimed to document lessons learnt during the South African seabird HPAI outbreaks, address epidemiological knowledge gaps and examine options for future outbreak response. It begins with a description of the 2018 and 2021/2022 outbreaks of HPAI in coastal birds and the lessons learnt from the outbreak responses.

To improve understanding of the epidemiology of HPAI in African Penguins, and inform future outbreak response, a cross-sectional survey was conducted across their breeding range in 2021 and 2022. This involved sampling of 530 adult penguins in breeding colonies and 382 penguins of varying ages, admitted to a rehabilitation centre. Possible risk factors, such as colony density, were linked with the detection of virus in penguins admitted for rehabilitation.

Vaccination is a potential option for managing HPAI in seabirds, though fully effective vaccines are not yet available and suitable formulations and vaccination protocols have not been explored. A randomised trial was conducted in captive African Penguins. It compared the magnitude and duration of antibody response to a conventional inactivated clade 2.3.4.4b H5N8 HPAI whole virus vaccine, with the response to a tobacco leaf-produced H5 hemagglutinin based virus-like particle (VLP) vaccine. The VLP vaccine induced significantly higher antibody titres by day 14 and one dose induced similar titres to two doses of the inactivated vaccine. VLP vaccines therefore provide an example of a vaccine technology that may help vaccination to become more feasible for wild birds.

The results of this research were used to compile preparedness plans for disease outbreaks generally, and HPAI specifically in South African coastal birds. Stakeholders were consulted and international workshops attended to identify information required by those involved in responding to an outbreak. It was challenging to obtain input during writing of the plans, but they contain the necessary background information, responsibilities of different stakeholder groups and activities to prepare for outbreaks and they discuss the options for response.

There is currently very little that can be done to stop or slow an HPAI outbreak in wild birds, so preparation for outbreaks, efficient use of resources and consolidation of accumulated knowledge is vital. This dissertation documents lessons learnt from responding to and planning for unprecedented seabird outbreaks in South Africa, which can be built on and refined for other scenarios. The mortality rate, attributed to HPAI, varied across African Penguin colonies, most likely due to high environmental viral load and proximity of breeding birds in some colonies. This information can be used to estimate risk, compile surveillance plans and prioritise potential mitigation measures. Adult African penguins appear more susceptible to the virus than juveniles, which has a relatively more serious population implications, and vaccination is one of the few potential tools to protect these valuable birds. This dissertation illustrates that avian influenza vaccines should be safe and effective in African Penguins and that technology is available that could make vaccination programs feasible, especially if less invasive application methods can be developed.

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Abbreviations

AGID	Agar gel immunodiffusion
AI	Avian influenza
AIV	Avian influenza virus
ARC-OVR	Agricultural Research Council's Onderstepoort Veterinary Research Institute
BCI	Body condition index
cELISA	Competitive enzyme-linked immunosorbent assay
CI	Confidence interval
DFFE	Department of Environment, Forestry and Fisheries
DIVA	Differentiation of infected and vaccinated animals
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FAQ	Frequently asked questions
Gs/GD	Goose-Guangdong
GIS	Geographic information systems
GMT	Geometric mean titre
GPS	Global positioning system
HA	Haemagglutinin
HAU	Haemagglutinating units
HI	Haemagglutination inhibition assay
HP	High pathogenicity
HPAI	High pathogenicity avian influenza
HPAIV	High pathogenicity avian influenza virus
IAV	Influenza A virus
ICU	Intensive care unit
IMS	Incident management system
IUCN	International Union for Conservation of Nature
LPAI	Low pathogenicity avian influenza
NA	Neuraminidase
NCD	Newcastle disease
NDV	Newcastle disease virus

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NGS	Next Generation Sequencing
OIE	World Organisation for Animal Health, founded as the OIE (acronym OIE used until May 2022)
OR	Odds ratio
PBS	Phosphate buffered saline
rRT-PCR	Real-time reverse transcription polymerase chain reaction
PCR	Polymerase chain reaction
PCV	Packed cell volume
PPE	Personal protective equipment
ppt	Parts per thousand
R0	Reproductive number
RBC	Red blood cell
RNA	Ribonucleic acid
SANCCOB	Southern African Foundation for the Conservation of Coastal Birds
SANParks	South African National Parks
SD	Standard deviation
SOP	Standard Operating Procedure
SPCA	Society for the Prevention of Cruelty to Animals
TSP	Total serum protein
VLP	Virus-like particle
WCPVL	Western Cape Provincial Veterinary Laboratory
WOAH	World Organisation for Animal Health (acronym WOAH used after May 2022)

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Chapter 1

Introduction

High pathogenicity avian influenza (HPAI) is a viral disease of birds, caused by Influenza A viruses from the family Orthomyxoviridae. HPAI viruses have specific sequences of amino acids in their surface proteins, which allow them to bind to tissue in a variety of organ systems and cause severe disease and high mortality rates in chickens, though effects on other species are variable. They arise from low pathogenicity avian influenza (LPAI) viruses through genetic mutation and reassortment that usually occurs during prolonged circulation in domestic poultry (Lee *et al.*, 2017, 2021). LPAI viruses occur naturally in wild waterfowl and members of the order Charadriiformes, which includes waders, gulls and terns. They usually cause subclinical infections or only mild disease as they are only able to infect the respiratory, reproductive and digestive tracts.

HPAI was primarily a disease of domestic poultry, with few recorded outbreaks or reservoirs in wild birds, until emergence of the Goose-Guangdong (Gs/GD) H5N1 HPAI virus lineage. It was first detected in China in 1996 and by 2005 was perceived as a global threat to poultry production and, following some zoonotic transmission, as a potential cause of a global human pandemic. This virus was also detected more frequently in wild birds, and caused more mortality than previous HPAI viruses (Gauthier-Clerc *et al.*, 2007).

The threat of HPAI receded slightly until the detection of the Gs/GD clade 2.3.4.4a H5N8 viruses in 2014, which reached North Africa and North America (Lee *et al.*, 2021). Clade 2.3.4.4b H5N8 travelled as far as South Africa in 2017, devastating the poultry industry and, in 2018, causing mass mortality in wild seabirds. The clade 2.3.4.4b Gs/Gd H5N1 virus emerged in 2020, possibly in Europe (Xie *et al.*, 2023), and reached South Africa by April 2021 (Abolnik *et al.*, 2023). It caused mass mortality in Endangered seabirds in South Africa from September to December 2021 and in Europe and the Americas from 2022 onwards (European Food safety Authority (EFSA) *et al.*, 2023; Harvey *et al.*, 2023), raising serious concerns about the conservation effects of the disease (Ramey and Handel, 2022). Additionally, H5N1 viruses have caused population-level impacts on marine mammals in South America (Campagna *et al.*, 2024) and continue to cause sporadic human infections (Bruno *et al.*, 2023).

South Africa experienced its first outbreaks of HPAI in chickens in 2017 but could base the response on existing contingency plans and on guidelines and publications from overseas. When mortality rates started to increase in seabirds, conservation authorities were completely unprepared and veterinarians who tried to assist could find few published guidelines. The only significant disease previously faced in South African seabirds was avian cholera, caused by *Pasteurella multocida* (Waller and Underhill, 2007). However, outbreaks were localised and the disease's epidemiology better understood. Decisions about HPAI response were based on available knowledge of general HPAI epidemiology and discussions between the different role- players, and guideline documents were developed one by one. The lessons learnt and networks formed were helpful in responding to the next outbreak in 2021 and in assisting others who were responding to seabird outbreaks elsewhere. However, there was still a lack of preparedness for disease outbreaks and awareness of management options and actions required, within and among conservation management authorities. There were frequent requests for written preparedness and contingency plans and regular training. There was also a need for a more structured and comprehensive surveillance system, to establish baseline mortality rates in seabirds and to allow early detection of abnormalities and a swift response.

Conservation concerns were first raised in southern Africa in 2018, when at least 100 endangered African Penguins (*Spheniscus demersus*) were estimated to have died from HPAI on South Africa's coast. By April 2019, at least 459 had died on three Namibian islands (Cannon, 2019). African Penguins occur only off the coast of southern Africa, in South Africa and Namibia, and the population has

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declined by 95% in the last 100 years (Crawford *et al.*, 2011; Makhado *et al.*, 2018). In 2019, there were less than 20 000 breeding pairs (Birdlife International, 2024). These were the first recorded avian influenza outbreaks in any species of penguin, although there was evidence that they could be infected, from Antarctic and sub-Antarctic species (Abad *et al.*, 2013; Barriga *et al.*, 2016; Hurt *et al.*, 2016, 2014). Nothing was known about the epidemiology or distribution of avian influenza virus in African Penguins, hampering conservation and management strategies.

Management of HPAI during the South African outbreaks in seabirds was limited to the removal of infected birds and to the restriction of human activities that could cause additional stress to the penguins and contribute to the mechanical transmission of the virus. However, there were frequent questions about other preventative methods such as vaccination. No HPAI vaccines were approved for use in South Africa at the time and there was no idea of how effective a vaccine would be in penguins, even if it could be applied successfully to a wild population.

The projects described in this dissertation aimed to address some of the shortcomings and knowledge gaps identified during the South African seabird HPAI outbreaks. First, Chapter 2 describes the existing literature relevant to understanding and responding to HPAI in seabirds. It provides general background to avian influenza viruses, and more detail on spread and impact of the recent Gs/Gd clade 2.3.4.4b viruses. It also outlines the existing understanding of avian influenza epidemiology in southern Africa. Chapter 2 goes on to describe current approaches to avian influenza surveillance and management in wild birds and background about avian influenza vaccination. African Penguin biology relevant to disease spread and research is covered, and, lastly, background on compiling preparedness plans for disease outbreaks in coastal birds.

Chapter 3 aims to consolidate the epidemiological observations made during the 2018 and 2021 South African seabird outbreaks of H5N8 and H5N1 HPAI respectively. Numbers of birds suspected to have died from the virus and laboratory-confirmed cases are reported, and comparisons are made between species, locations and outbreaks. The Chapter also documents the experience gained and the lessons learnt during the outbreak response, focussing on communication, measurement of the outbreak, response in the wild and management in rehabilitation centres. It provides recommendations for future outbreaks in South Africa and elsewhere.

Chapter 4 describes a survey in African Penguins, conducted from 2021 to 2022, aimed at estimating the prevalence of avian influenza virus and antibodies in African Penguins. Adult penguins were sampled in breeding colonies, in order to perform serology and virus screening via rRT-PCR. Additionally, the proportion of infected penguins admitted to a rehabilitation centre was estimated via rRT-PCR testing and the relationship between infection status and clinical signs, age, sex, body mass and origin were investigated.

The objective of Chapter 5 is to investigate the potential for HPAI vaccination in seabirds, by comparing the magnitude and duration of antibody response to, and assessing the safety of, two clade 2.3.4.4b high pathogenicity avian influenza H5 vaccines in African Penguins. The chapter also explores the logistics of wildlife vaccination programs in general and in seabirds specifically, to gauge their feasibility.

Chapter 6 describes the process of and lessons learnt from compiling a preparedness plan for disease outbreaks in South African seabirds. An HPAI-specific plan was also drafted and a site-specific plan for HPAI on sub-Antarctic Marion Island.

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Chapter 2

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1. Introduction

Infectious disease control in animals and humans has advanced dramatically in the last century, with increasingly detailed knowledge of different pathogens and how they survive, multiply and spread. Understanding of hygiene and the development of antibiotics, antivirals, disinfectants and vaccines has allowed for increasing productivity and longevity in humans and animals. However, understanding the Epidemiological Triangle and the interaction between pathogen, host and environment is more important for some diseases than others. These are often diseases where disinfectants cannot be used or work to their full potential or where there is innate or adaptive resistance to antibiotics, antivirals and vaccines, or simply no feasible way of applying them. High pathogenicity avian influenza is one of these complex diseases. There is interplay between vector species, possibly carrying the virus subclinically and species in which the virus causes high morbidity and mortality rates. The environment affects behaviour of vector species and virus survival. Lastly, there may be incidental hosts, like humans, that may nonetheless become victims of a pandemic. This could happen after a few genetic mutations and virus reassortments, which happen so easily in avian influenza viruses.

High pathogenicity avian influenza viruses are now a threat to biodiversity and conservation, of both birds and mammals. The latest dominant Goose/Guangdong clade 2.3.4.4b H5 viruses have proven capable of spreading round the globe in a matter of months, decimating domestic and wild birds and marine mammals along the way. There is also the constant possibility of a pandemic, if the virus gains the capability to spread from human to human. This dissertation describes the recent, unprecedented effects of HPAI H5N8 and H5N1 viruses on South African seabirds and the outbreak response efforts. The dissertation then investigates HPAI epidemiology in African Penguins and explores vaccination as an option for their protection against HPAI. Lastly, it describes the compilation of preparedness plans, to ensure efficient response to future outbreaks.

To understand HPAI virus epidemiology and examine ways to mitigate its effects, one needs to understand the virus and its structure and capabilities, its interaction with environmental factors and the species in which it may be transported around the globe. These topics comprise the first two sections of this chapter. Prior knowledge of southern African low pathogenicity avian influenza (LPAI) epidemiology is then described, followed by accounts of the spread and current understanding of the clade 2.3.4.4.b HPAI viruses. To further investigate and combat these viruses, there is a need for understanding of established surveillance methods and programs for wild birds and current guidelines for outbreak management. Vaccination is one of the few tools that can combat avian influenza spread, when hygiene and biosecurity fail or are not feasible. This chapter provides background on current avian influenza vaccine technology, methods to measure efficacy and considerations for designing wildlife vaccination programs. Investigation of avian influenza epidemiology in African Penguins requires background about their life history and ecology which is supplied in Section 9. Lastly, this chapter covers background and guidelines for preparedness plans for wild bird HPAI outbreaks and describes Citizen Science as a tool that can be used to improve surveillance and early warning systems for HPAI outbreaks.

2. Avian influenza

Avian influenza is a viral disease of birds. High pathogenicity avian influenza (HPAI) strains cause severe illness and high mortalities in chickens and can have variable effects in other avian species. Low pathogenicity viruses generally cause few or mild clinical signs, related to infection of the respiratory and digestive tracts.

Avian influenza viruses are classified according to their surface proteins, haemagglutinin (H) and neuraminidase (N), which are numbered according to the serotype e.g. H5N8. The haemagglutinin protein attaches to host cell receptors, in order to enter the cell, and neuraminidase is involved with

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the efficient release of the virus from the host cell (Suarez, 2017). HPAI viruses, usually H5 and H7, have a specific amino acid sequence (cleavage site) in the haemagglutinin gene that gives the haemagglutinin protein the ability to facilitate entry to a wider variety of host tissues in chickens and therefore increases pathogenicity. Low pathogenicity avian influenza (LPAI) viruses can only infect cells of epithelium in the respiratory and digestive tracts.

The haemagglutinin and neuraminidase proteins are encoded by two of the eight gene segments that comprise the genome of an avian influenza virus. Because of this segmentation of the genome, infection of a bird with two or more different avian influenza viruses can result in swapping of these segments and the production of a new virus. This is referred to as genetic reassortment. Poor proofreading during replication also results in a high rate of random genetic mutations and the potential for viruses to gain new capabilities rapidly.

Avian influenza viruses (AIV) are spread by direct contact between infected and healthy birds, or through indirect contact with contaminated water or other materials or objects (fomites). The virus is present in the faeces and respiratory secretions of infected birds, but the quantities of virus secreted and the dominant route of secretion vary with bird species and virus strain. The faecal-oral route is believed to be important for infection of waterfowl but aerosol spread and the respiratory route may be more important for infection of poultry with some high pathogenicity strains and for infection of terrestrial wild birds. Additionally, raptors and scavengers may be infected from contaminated carcasses (Fouchier and Munster, 2009; Shriner and Root, 2020; Spickler, 2022).

Survival of avian influenza viruses in the environment is prolonged by the presence of organic material, as well as lower temperatures, more moisture and protection from sunlight (Sims *et al.*, 2017b). H5 HPAI viruses were found to last more than three months in liquid poultry manure at approximately 0°C and only four days at 25-32°C (Sims *et al.*, 2017b), demonstrating the effect of temperature. Microbial activity also plays an important role. LPAI (H5N7) virus survival increased from an estimated nine days in filtered brackish water (8 ppt salinity, pH 7.1), at 4°C, to 263 days when the water was autoclaved before the virus was added (Nielsen *et al.*, 2013). In addition, virus survival is affected by salinity and pH (Brown *et al.*, 2009). An HPAI H5N1 virus survived approximately 5 months in distilled water (pH 7.2, 0 ppm salinity) at 17°C (Brown *et al.*, 2007), demonstrating extended survival at neutral pH and low salinity, in the absence of biological material. A study involving LPAI viruses showed that increasing salinity or pH shortened virus survival times in distilled water (Sims *et al.*, 2017b; Stallknecht *et al.*, 1990), as did lowering pH (Brown *et al.*, 2009). Salinity and pH may also interact. Stallknecht *et al.* (1990) found that LPAI viruses were relatively stable in slightly alkaline water (pH 7.2 to 8.2) at low salinity but with higher salinity the virus survived longer under more acidic conditions, with a peak between pH 6.6 and 7.4 at 30ppt salt (seawater salinity). There may also be an interaction between pH and temperature, with lower pH being more likely to inactivate the virus at higher temperatures. Salinity also interacts with temperature: higher salinity (measured up to 30 parts per thousand (ppt)) may prolong virus survival at low temperatures (Brown *et al.*, 2007; Martin *et al.*, 2018). This was demonstrated by Nielsen *et al.* (2013) who estimated that an LPAI (H5N7) virus would survive approximately nine days in brackish water (8 ppt salinity) at 4°C but 15 days in seawater (20 ppt). At 17 and 25°C, however, the virus was estimated to last less than 3 days in both brackish and seawater. The authors note that the seawater was less saline than the average, which is 35 ppt, but this is one of the few studies that estimates virus survival in seawater. It indicates that virus survival will be relatively short in temperate climates such as Cape Town's, with seawater temperatures ranging between approximately 14°C and 21°C (https://www.seatemperature.org/). However, it is still not clear if the virus will be destroyed quickly enough to prevent transmission in seawater, especially in small volumes of water, such as rock pools. Virus survival may depend on other factors, such as the level of contamination with infected faeces. Varying soil and fresh water pH and contamination may also dictate varying virus survival times, and risk to wild birds, at different sites.

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3. Avian influenza virus hosts

The natural, adapted hosts of low pathogenicity avian influenza (LPAI) viruses are believed to be wild water birds, especially ducks and geese (water fowl) in the Order Anseriformes, Family Anatidae and gulls and terns from the Order Charadriiformes, Family Laridae (Bodewes and Kuiken, 2018). Waders (Charadriiformes, Charadriidae) are often said to play a significant role in avian influenza epidemiology but this has been contested and will be discussed further below waders (Caron *et al.,* 2017; Fouchier and Munster, 2009).

3.1. Waterfowl (Order Anseriformes)

There is a high prevalence of low pathogenicity avian influenza viruses (LPAIV) in Mallards (*Anas platyrhynchos*) in autumn in North America and Europe, when juvenile, immunologically-naïve birds gather for the autumn migration (Bodewes and Kuiken, 2018). The large quantity of virus shed via the faeces of ducks, and long survival periods in fresh water, especially at lower temperatures and salinity, contribute to faecal-oral transmission of AIV, with environmental contamination possibly playing an important role (Bodewes and Kuiken, 2018). However, respiratory excretion of AIV can also be important in particular species or non-aquatic environments (Fouchier and Munster, 2009). Waterfowl species can shed LPAI, or even HPAI, virus for long enough, without being clinically affected, to transport it long distances during migration and spread it at aquatic stopover sites or the final destination. Genomic sequencing has confirmed that this occurs with HPAI virus. Wild waterfowl have therefore been recognised as efficient long-distance vectors of HPAI virus.

Hill *et al.* (2019) provide one of the first descriptions of repeated HPAIV outbreaks in an individual population of wild birds. This assists with predicting the effects of consecutive outbreaks on other species. A population of approximately 800 Mute Swans (*Cygnus olor*) in the United Kingdom was infected by both HPAI H5N8 in 2016 and H5N6 in 2017 and they were intensively monitored and sampled. Both viruses appear to have been introduced and caused mortality during the time of peak autumn numbers of migratory birds, though few other birds at the site appear to have succumbed to either virus, except for two Canada Geese (*Branta Canadensis*) and a Common Pochard (*Aythya farina*). Immunological status was identified as an important contributor to a higher survival rate during the 2017 H5N6 outbreak: juvenile swans that were not alive during the previous outbreak were more likely to die and birds that died did not have H5 antibodies. In contrast, adults present during the previous outbreak were H5 seropositive and more likely to survive the 2017 outbreak. Survival rates may therefore depend on population composition, which is in turn affected by lifespan. Another finding was a relatively high estimated reproductive number (R_0), of approximately 2.5, for both outbreaks. High population density, linked to supplementary feeding, as well as the high proportion of naïve juveniles, were cited as possible causes. Additionally, though not found to be significant, older juveniles and those with lower body mass appeared more likely to survive. These are therefore factors to consider when conducting risk analyses and assessing possible migration measures for susceptible populations.

3.2. Waders (Family Charadriidae)

Though a wide variety of subtypes have been detected in waders (Charadriiformes, Charadriidae), especially in North America, many studies were performed in the Delaware Bay area and extrapolation of findings to other areas may not be suitable (Caron *et al.,* 2017; Fouchier and Munster, 2009). Munster *et al.* (2007) did not find any evidence that waders played an important role in avian influenza epidemiology in Europe. Maxted *et al.* (2012) postulated that the extremely high densities (up to 67 birds/m^2) of Ruddy Turnstones (*Arenaria interpres*) during spring migration at Delaware Bay, and possibly the stress of migration and refuelling, may be the reason for annual LPAI epidemics at the site. The original source of the virus was never clear, but infection and recovery occurred onsite and seroprevalence increased from less than 50% to nearly 100% over the stopover period of

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approximately a month, before departure. This is the only time these birds congregate in large numbers and at this density, and, even if a few birds bring the virus from elsewhere (rather than Delaware Bay providing a source), this may provide the only opportunity for such explosive spread (Maxted *et al.*, 2012). Though the role of waders in avian influenza epidemiology is still in question, the studies at Delaware Bay again highlight the role of bird aggregation in virus transmission rates.

3.3. Seabirds (including Laridae)

To assess the role of seabirds in avian influenza epidemiology, Lang *et al.* (2016) reviewed previous studies of LPAIV in seabirds, excluding gulls. They found overall virus detection rates to be very low (1%), though rates were much higher for some species. For example, of the Northern Fulmar (*Fulmaris glacialis*) and Lesser Noddy (*Anous tenuirostris*) tested, 11 and 5.1% respectively were found to be infected. In contrast, antibodies were detected in 61% of species tested and the seropositive proportion was higher than 5% in most affected species. Species with more than 20% seropositive included Atlantic Puffin (*Fratercula arctica*), Common Murre (*Uria aalge*), three noddy (*Anous*) species, Southern Giant Petrel (*Macronectes giganteus*), and Macaroni Penguin (*Eudyptes chrysolophus*). Some species were also identified as being the source of a greater variety of virus subtypes, especially murres but also Lesser Noddy, Wedge-tailed Shearwater (*Puffinus pacificus*), penguins and terns. The authors commented, however, that surveillance in seabirds is challenging and has not been done methodically or consistently. Low pathogenicity viruses may circulate more successfully in these seabird species with higher seropositivity rates, which may play a role in virus spread to other species. However, spread of HPAI viruses may not occur in the same manner if some species suffer higher morbidity and mortality, as well as infection rates and are therefore less mobile.

Arnal *et al.* (2015) reviewed research on avian influenza viruses in the bird family Laridae, predominantly gulls, and compared known epidemiology to that in Anatidae. The respiratory tract may be the more important route of infection in gulls, as the digestive tract is in Anatids. This may be linked to differences in epithelial receptor distribution and also explain subtypes found predominantly in gulls, i.e. H16 and H13 (Lang *et al.*, 2016). Direct transmission due to aggregation in dense breeding colonies may also be a more feasible route of transmission for gulls, rather than indirect transmission, via contaminated fresh water, as it is in ducks and geese (Arnal *et al.*, 2015). Coastal and marine environments with high salinity may also make environmental transmission less likely (Brown *et al.*, 2007; Martin *et al.*, 2018; Stallknecht *et al.*, 1990. Peaks in virus prevalence in gulls are less defined and predictable than the late summer and winter peaks, coinciding with migration, in waterfowl in the northern hemisphere. This may be linked to the differing conditions that favour transmission; however, sample sizes also may not have been large enough to detect lower peaks in immature gulls. Peaks in virus prevalence may also be less defined in gulls due to their longer lifespan and opportunity to develop better immunity, which can also be transferred to offspring in the egg (Arnal *et al.*, 2015). Other seabird species, which breed under similar conditions and also have long lifespans, may have similar transmission dynamics to gulls

Despite the differences from waterfowl, gulls infected with avian influenza may also be a source of virus for poultry and humans, through different routes of interaction. The Laridae family may also contribute to intercontinental virus spread. Gulls adapt well to urban conditions and are skilled scavengers, which may bring them into close contact with domestic birds and humans and provide an opportunity for virus transmission. Long-distance migrations of Larids may also lead to virus spread between continents. Viruses that are reassortants of American and Eurasian strains have been detected in Larids and this may be evidence of that the group is capable of contributing to intercontinental spread (Arnal *et al.*, 2015).

Boulinier (2023) discussed the possible role that seabird movements between colonies play in the spread of AIV. Seabirds breed in dense colonies, that are expected to favour virus transmission, but

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Table 2.1: Results of previous studies in which wild penguins were tested for avian influenza virus and antibodies

Penguin Species*	**Location**	**Method**	**n**	**% pos.**	**Year**	**Reference**
Adélie	Antarctic Peninsula (AP)	cELISA	22	4.5	2010-2013	de Seixas *et al.* (2022)
Adélie	Antarctic Peninsula (AP)	rRT-PCR	45	0	2010-2013	de Seixas *et al.* (2022)
Adélie	Antarctic Peninsula (AP)	"serum samples positive for influenza"	138	3.6	2015-2016	Barriga *et al.* (2016)
Adélie	Admiralty Bay (AP)	rRT-PCR (cloacal/tracheal)	110 ad. 40 chicks	0 2.5	2013	Hurt *et al.* (2014)
Adélie	Rada Covadonga (AP)	rRT-PCR (cloacal/tracheal)	111 ad. 40 chicks	5.4 2.5	2013	Hurt *et al.* (2014)
Adélie	Admiralty Bay (AP)	cELISA	110 ad. 40 chicks	16.7 0	2013	Hurt *et al.* (2014)
Adélie	Rada Covadonga (AP)	cELISA	111 ad. 40 chicks	27.3 0	2013	Hurt *et al.* (2014)
African	South Africa, Namibia	HI (H5 and H7)	439	0	2007-2013	Parsons *et al.* (2016)
Chinstrap	Antarctic Peninsula (AP)	cELISA	94	32	2010-2013	de Seixas *et al.* (2022)
Chinstrap	Antarctic Peninsula (AP)	rRT-PCR	123	8.9	2010-2013	de Seixas *et al.* (2022)
Chinstrap	Livingston Island (AP)	cELISA	17	0	2009	Abad *et al.* (2013)
Chinstrap	Antarctic Peninsula (AP)	rRT-PCR (cloacal/tracheal)	493	0.2	2015	Hurt *et al.* (2016)
Chinstrap	Cape Shirreff (AP)	cELISA	104	10	2015	Hurt *et al.* (2016)
Chinstrap	King George Island (AP)	cELISA	100	31	2015	Hurt *et al.* (2016)
Chinstrap	Kopaitik Island (AP)	cELISA	103	14	2015	Hurt *et al.* (2016)
Galápagos	Galápagos Islands	AGID	75	0	2004	Travis *et al.* (2006)
Gentoo	Livingston Island (AP)	cELISA	26	0	2009	Abad *et al.* (2013)
Gentoo	Antarctic Peninsula (AP)	rRT-PCR (Cloacal/faeces)	195 81	0 0	2014	Hurt *et al.* (2016)
Humboldt	Punta San Juan, Peru	AGID	61	0	1992-1994	Smith *et al.* (2008)
King	Marion Island	cELISA	27	0	2011	Abad *et al.* (2013)
Macaroni	Marion Island	cELISA	33	42	2011	Abad *et al.* (2013)
Magellanic	Argentina	AGID	454	0	1994-2008	Uhart *et al.* (2020)
Northern Rockhopper	Gough Island	cELISA	19	5	2009	Abad *et al.* (2013)
(Southern) Rockhopper	Santa Cruz, Argentina	Serology ("according to OIE")	45	0	1994	Karesh *et al.* (1999)
Southern Rockhopper	Marion Island	cELISA	18	11	2011	Abad *et al.* (2013)
Adélie	Antarctic Peninsula (AP)	rRT-PCR	513	0	2015-2016	Barriga *et al.* (2016)
Chinstrap				(8)	2015-2016	Barriga *et al.* (2016)
Gentoo				(13)	2015-2016	Barriga *et al.* (2016)

*Adélie: *Pygoscelis adeliae*, Chinstrap: *P. antarcticus*, Galápagos: *Spheniscus mendiculus*, Gentoo: *P. papua*, Humboldt: *S. humboldti*, King: *Aptenodytes patagonicus*, Macaroni: *Eudyptes chrysolophus*, Magellanic: *S. magellanicus*, N. Rockhopper: *E. moseleyi*, S. Rockhopper: *E chrysocomes*.

AGID: agar gel immunodiffusion, cELISA: competitive enzyme-linked immunosorbent assay, rRT-PCR: real-time reverse transcription polymerase chain reaction, HI: haemagglutination inhibition assay

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risk of transmission should be much lower during extended times at sea. However, specific behaviour at sea, such as aggregation during feeding, predation or stealing fish from other birds (kleptoparasitism), could create a higher risk for virus spread. Breeding adults tend to breed at the same colony every year, so are not expected to play a major role in virus spread. However, predators and scavengers such as gulls and skuas could play a role in the spread between colonies, and are already implicated in the spread of avian cholera (*Pasteurella multocida;* Gamble *et al.*, 2020). Failed breeders and pre-breeders may also visit multiple colonies and generally move more widely (Lamb *et al.*, 2023). More tracking of individuals like pre-breeders, further study of virus phylodynamics, more serology, examination of contact networks between and within species and more use of mark-recapture studies were recommended to improve understanding of AIV spread between colonies.

3.3.1. Penguins

Evidence of previous avian influenza infection has been detected in most Antarctic and sub-Antarctic penguin species. The proportion of seropositive samples ranged from 0% to 42%, the latter in 33 Macaroni Penguins (Table 2.1; Abad *et al.*, 2013; Barriga *et al.*, 2016; de Seixas *et al.*, 2022). Low pathogenicity avian influenza virus has also been identified in Adélie (*Pygoscelis adeliae*), Gentoo (*Pygoscelis papua*) and Chinstrap (*Pygoscelis antarcticus*) Penguins on the Antarctic Peninsula (Barriga *et al.*, 2016; de Seixas *et al.*, 2022; Hurt *et al.*, 2016, 2014) and reports of clinical epidemics or clinical signs associated with LPAI viruses are very sparse or anecdotal (Barriga *et al.*, 2016; Morgan and Westbury, 1981). The proportion of positive samples varied from 0.2% (1/143; Hurt *et al.*, 2014) to 8.9% (de Seixas *et al.*, 2022) and the only abnormalities recorded in affected chicks were weakness and depression (Barriga *et al.*, 2016). There is evidence that some viruses have circulated on the Peninsula for decades since introduction from further north (Hurt *et al.*, 2014).There is evidence of local reassortment (Hurt *et al.*, 2014) and links with America and Eurasia (Barriga *et al.*, 2016; de Seixas *et al.*, 2022). Though there was no evidence of HPAI virus introduction, transfer of avian influenza virus to the Antarctic and sub-Antarctic, and subsequent circulation, was shown to be possible.

Further north, Surveys of Rockhopper (*Eudyptes chrysocomes)* and Magellanic Penguins (*Spheniscus magellanicus*) have been conducted in Argentina (Karesh *et al.*, 1999; Uhart *et al.*, 2020), of Humboldt Penguins in Peru (Smith *et al.*, 2008), African Penguins in South Africa and Namibia (Parsons *et al.*, 2016) and Galapagos Penguins (Galapagos Islands: Travis *et al.*, 2006). None of these studies found AIV antibodies in penguins north of the sub-Antarctic (Table 2.1) but avian influenza researchers consistently report the need for robust, longer-term surveillance (Barriga *et al.*, 2016; Chang *et al.*, 2009; Parsons *et al.*, 2016).

de Seixas *et al.* (2022) state that birds were sampled opportunistically, and this is assumed to be the case for all penguin studies, but they are the only group to mention sample selection method. The proportion of positive samples therefore cannot be interpreted as population prevalence, though the detection of avian influenza antibodies and virus has still provided valuable information on the ability of the viruses to reach and circulate in Antarctica and to infect penguins as a group.

4. Avian influenza in southern Africa

4.1. Low pathogenicity avian influenza viruses and their ecology in southern Africa

Low pathogenicity avian influenza (LPAI) viruses have been detected in South Africa for years, circulating harmlessly in wild birds and being regularly detected in commercial ostriches, which have regular contact with wild birds. Research in southern Africa has found evidence that wild waterfowl are the natural hosts, as elsewhere (Abolnik, 2010; Caron *et al.*, 2011; Pfitzer *et al.*, 2000). Seven of the sixteen resident southern African anatid species (ducks and geese) have been the source of isolated virus, and these have generally been the more abundant species, so they were more likely to be sampled (Abolnik *et al.*, 2016; Hockey *et al.*, 2005). The host range may therefore be even wider

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than the species in which virus has been detected. Cumming *et al.* (2008) performed a risk analysis and identified Egyptian Geese (*Alopochen aegyptiaca*) and Red- Billed Teal (*Anas erythrorhynchus*) as being the most likely anatid species to carry avian influenza. However, LPAI virus has also been isolated from African Sacred Ibis (*Threskiornis aethiopicus*) and Hadeda Ibis (*Bostrychia hagedash*) (order *Ciconiiformes)* (Pfitzer *et al.*, 2000). A variety of other species have been found to be infected via molecular methods, including Swift Terns in Mossel Bay, South Africa (Abolnik *et al.*, 2010) and others from the order Charadriiformes, and members of the orders Passeriformes (perching birds) and Coraciiformes (kingfishers, bee-eaters, rollers) in the Manyame Catchment area of Zimbabwe (Caron *et al.*, 2011).

The exact mechanisms of virus introduction and spread in southern Africa are still unknown but LPAI virus appears to have been introduced from both North America (Abolnik, 2007) and Eurasia (Abolnik *et al.*, 2006), and from distant African countries like Nigeria (Gaidet *et al.*, 2008; Abolnik *et al.*, 2010). Although some species of southern African waterfowl may move considerable distances in Africa, they are not considered truly migratory (Oatley and Prys-Jones, 1986) and therefore not solely responsible for the introduction of avian influenza viruses from the northern hemisphere. Gaidet *et al.* (2007) found AIV in Palearctic migratory ducks; garganey (*Spatula querquedula*), northern pintail (*Anas acuta*), green- winged teal (*A. crecca*), and northern shoveler (*A. clypeata*), in West Africa, but these species only occur in southern Africa as uncommon vagrants (or escaped from captivity) (Hockey *et al.*, 2005). However, there could be relay transmission where ducks moving within Africa could make contact with ducks from southern Africa and with Palearctic- breeding ducks overwintering at wetlands in North Africa (Abolnik *et al.*, 2010). Gaidet *et al.* (2008) showed that a White-faced Whistling duck (*Dendrocygna viduata*) travelled 655 km over 47 days, while infected with HPAI. Gaidet *et al.* (2010) tracked wildfowl from eight countries in Europe, Asia & Africa from 2006 to 2009 and found that individual migratory waterfowl could disperse HPAI H5N1 over distances of up to 2900 km. They are, however, unlikely to do so because the stopovers are longer than the expected period of infectivity. Therefore, relay transmission is more likely. This may explain the transmission pathways, but predicting virus introduction is still difficult. Intra-African waterfowl movements are relatively unpredictable, unlike strongly seasonal patterns in the Northern hemisphere that appear to dictate peaks of AIV infection (Cumming *et al.*, 2012; Gaidet *et al.*, 2012a). African waterfowl movements respond to more short-term climatic factors like rainfall (Cumming *et al.,* 2008).

Long-distance migratory waders (Sub-order Charadrii) have also been implicated as possible vectors of avian influenza viruses from Europe and Asia to Africa. Gaidet *et al.* (2007) found LPAI in Eurasian waders, including Curlew Sandpiper (*Calidris ferruginea*), Ruff (*Philomachus pugnax)* and Spotted Redshank (*Tringa erythropus*), in Mali and Tunisia, which indicates a possible route of introduction, as waders are the largest group of Eurasian water bird migrants. However, the role of waders as significant vectors of avian influenza virus has been questioned, since they usually appear have a low virus prevalence; less than 2% (Gaidet *et al.*, 2012b).

Caron *et al.* (2012) questioned whether the role of non-anatid and charadrid species in avian influenza virus transmission might have been underestimated, especially as bridges to domestic poultry. Caron *et al.* (2014) identified Barn Swallows (*Hirundo rustica)*, Red-billed Queleas (*Quelea quelea)* and Cattle Egrets (*Bubulcus ibis)* as possible bridge species in the Manyame catchment area and 1% of Red-billed Queleas and 3% of Barn Swallows sampled were AIV positive. Caron *et al.* (2017) made further studies of AIV in swallows but struggled to explain a sufficiently reliable route of infection for these birds.

4.2. HPAI history in South Africa

The first ever record of AIV isolation from wild birds, worldwide, was from Common Terns (*Sterna hirundo*) that died in April 1961, in South Africa. Deaths occurred only in the one species, along the southern coast from Lambert's Bay on the west coast to Gqeherha (Port Elizabeth; Rowan, 1962), a similar distribution to reported deaths due to clade 2.3.4.4b HPAI (H5N8) virusin 2018. In 2018, this

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had been the only other recorded outbreak of avian influenza in coastal birds, and possibly in any wild bird population in South Africa. The virus was first called "tern virus" but was later identified as HPAI H5N3 (Abolnik, 2010). Rowan (1962) described the outbreak and Becker (1963) described the morphology of the virus and classified it as Influenza A in 1966. He also suggested a connection between outbreaks of AI in domestic chickens and contact with coastal birds, in Scotland. However, this connection may have been made only because one of the few previous HPAI outbreaks was in Scotland (Lee *et al.*, 2021).

Before 2017, the only HPAI outbreaks in domestic birds in South Africa had been in commercial ostriches and the low to high pathogenicity conversion apparently occurred in that species (Abolnik *et al.*, 2010). HPAI (H5N2) ostrich outbreaks occurred in 2004, 2006 and 2011, but the three strains were unrelated (Abolnik *et al.*, 2016) and did not appear to cause illness in chickens when infected experimentally (Abolnik, 2010). Low to high pathogenicity conversion is a non-gallinaceous species is unusual (Lee *et al.*, 2021) but there was no evidence that the viruses had been introduced from another country or continent.

5. Clade 2.3.4.4b high pathogenicity avian influenza (HPAI)

Avian influenza came to the fore as a threat to global poultry in 1996 with the high pathogenicity avian influenza A/Goose/Guangdong/1/96 (GsGD) H5N1 virus that emerged in China. It had reached 53 countries by 2006 and by December 2020 there had been 862 human cases and 455 deaths (53% case fatality rate) (World Health Organization, 2020). All had been linked to exposure to poultry and this was the first time that avian influenza was recorded to be transmitted directly to humans. Previous outbreaks, like the 1918 "Spanish Flu" only had RNA segments from avian influenza-like viruses (Taubenberger, 2006).

Over the years, the GsGD virus has diversified into multiple lineages or clades (Van Den Brand *et al.*, 2018) and panzootics have occurred as multiple intercontinental waves (Sims *et al.*, 2017a). The viruses have become progressively more widely spread and have affected a greater variety of wild birds (Klaassen and Wille, 2023). Wild birds also appear to both play an increasing role in the global spread of the viruses, while also being increasingly affected. HPAI is no longer only a poultry disease (Bodewes and Kuiken, 2018)

5.1. Clade 2.3.4.4b H5N8 viruses (2016 to 2018)

Clade 2.3.4.4 group A H5N8 caused outbreaks in South Korea in 2014 and then in other countries in Asia, Europe and North America (the third intercontinental HPAI wave). This was the first time since 2005, and since the original Goose Guangdong H5N1 outbreaks, that a single strain had spread over so large an area. It was also found that the haemagglutinin seemed to occur with an unusual variety of neuraminidase subtypes (The Global Consortium for H5N8 and Related Influenza Viruses, 2016). These viruses, however, appeared to cause largely asymptomatic infections in wild birds, compared to the first wave, which was lethal for a large number of birds from a variety of species, and the fourth wave, which, at the time, was the most severe in terms of numbers of wild birds and farms affected (Sims *et al.*, 2017a).

Clade 2.3.4.4 group B (2.3.4.4b) H5N8 was first detected in May/June 2016 on Uvs-Nuur Lake in the Republic of Tyva in Russia, on the northern border of Mongolia. Infected species included Black-headed Gulls (*Chroicocephalus ridibundus*), Great Cormorants (*Phalacrocorax carbo*), Grey Herons (*Ardea cinerea*), Great Crested Grebes (*Podiceps cristatus*), an unidentified duck, and a Common Tern (*Sterna hirundo*) (Lee *et al.*, 2017; Napp *et al.*, 2018). Few deaths were observed, unlike the 4 000 deaths caused by H5N1 in 2006 (Sims *et al.*, 2016). A very similar virus to Lake Uvs-Nuur was found at Qinghai Lake, China, 1600 km to the southeast, in May 2016 (Li *et al.*, 2017; Sims *et al.*, 2017a). One

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hundred and fifty-five bird carcasses were found over 15 days, comprising 124 Bar-headed Geese (*Anser indicus*), 17 Brown-headed Gulls (*Larus brunnicephalus*), and 14 Great Black-headed Gulls (*Ichthyaetus ichthyaetus*). HPAI H5N8 virus was detected in samples from all species via virus isolation, an unspecified subtyping method and whole genome sequencing.

The virus proceeded to spread across Asia and Europe and caused deaths in wild birds in the European Union (EU) mainly between October 2016 and February 2017 (European Union Reference Laboratory for Avian Influenza (EURL), 2018, 2017). Nine events were counted in which more than 50 birds were found dead, and 12828 birds were tested in 2016 and 19543 in 2017, compared to an annual average of 6460 over the previous 5 years (Brouwer *et al.*, 2017). Forty percent of outbreaks in wild birds in Europe involved swans (compared to 70% in 2006 H5N1), and Mallards and Tufted Ducks (*Aythya fuligula*) were the next most commonly involved, though with far fewer outbreaks (91 and 58 outbreaks of 955 respectively) (Napp *et al.*, 2018). The order Charadriiformes, mostly gulls, accounted for 11% and 7% of sick and dead wild birds tested in the EU in 2016 and 2017 respectively and 13.5% and 4% were positive for H5N8. One of 30 Common Terns tested in 2017 was positive and other positive Charadriiformes were one sandpiper (*Tringa spp.*) and two curlews (*Numenius spp.)* (European Union Reference Laboratory for Avian Influenza (EURL), 2018, 2017).

Kleyheeg *et al.* (2020) described the H5N8 outbreaks in wild birds and the response in the Netherlands in autumn-winter 2016/2017. They recorded 13590 carcasses and tested 255 (1.9%), of which 84 (33%) tested H5N8 positive, likely via PCR. Approximately half the carcasses were Tufted Ducks and Eurasian Wigeons (*Anas penelope*), and a large proportion of the remainder were listed only as unidentified waterfowl or unidentified birds. Mortalities in diving ducks were recorded as more than 2 000 times higher than the average calculated from mortality data from the Sovon database, of the Dutch Center for Field Ornithology. Their work demonstrated the difficulty in confirming infection during mass mortality events but showed that relative susceptibility of different species can be estimated and that baseline mortality figures are invaluable.

Clade 2.3.4.4b H5N8 reached Africa (Egypt) in November 2016. It was detected in Tunisia and Nigeria and then in Uganda in early January 2017 in White-winged Black Terns *(Chlidonias leucopterus*) on Lake Victoria. There was 60% mortality in this species (FAO, 2017; Ndumu *et al.*, 2018) and the virus was also detected in three Palaearctic species: two dead Eurasian Coots (*Fulica atra*) one dead Eurasian Wigeon and an asymptomatic (live) Common Teal (*Anas crecca*), and also in a grey-headed gull (*Chroicocephalus cirrocephalus*) (Khomenko *et al.*, 2018). Fusaro *et al.* (2019) postulate that West Africa was the source of both the Ugandan and South African outbreaks, though the West and East African outbreaks occurred at similar times and direction of spread could not be verified.

The virus was detected in Zimbabwe in May 2017 on a broiler breeder chicken farm and in the Mpumalanga Province of South Africa in broiler breeders on the 21st of June 2017. This was the first outbreak of HPAI in chickens recorded in South Africa. HPAI (H5N8) was first detected in the Western Cape province of South Africa in early August, on an ostrich farm on the south coast. The outbreaks in commercial chickens started later in August, near Cape Town. The first wild bird that tested positive was a Guinea Fowl (*Numida meleagris*) near Swellendam, near the south coast, in mid-August (Abolnik *et al.*, 2019).

Outbreaks continued to occur at a high rate in South African chickens, ostriches and wild birds, mostly found inland, until late October 2018. Approximately 70% of about 4.2 million commercial layer chickens in the Western Cape were lost and 66% of the approximately 280 ostrich farms were placed under quarantine. Wild bird species confirmed positive included a pigeon, Spur-winged Goose (*Plectropterus gambensis*), Guinea Fowl (three), dove (two), Blue Crane (*Anthropoides paradiseus*), House Sparrow (*Passer domesticus*), owl, Peregrine Falcon (*Falco peregrinus*) (two), Pied Crow (*Corvus albus*) (three), Sacred Ibis (*Threskiornis aethiopicus*), Black-headed Heron (*Ardea melanocephala*) and

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an Egyptian Goose (*Alopochen aegyptiaca*). There was then a lull until mid to late December, when reports of increased mortality in terns started. The last virus detection in domestic birds in the Western Cape was in January 2018, but further outbreaks occurred in the northern parts of the country, in domestic species, between February and June. These were, however, found to be due to a new introduction (Abolnik, 2019).

Abolnik *et al.* (2019) performed full genome sequencing on forty viruses obtained from commercial ostriches, terrestrial wild birds and poultry between June and October 2017. They found five different genetic variants but only one was responsible for the southern Cape outbreaks. They speculated that West Africa may have been the main source of virus for South Africa, with reference to Khomenko *et al.* (2018). They found evidence of reassortment with wild bird LPAI viruses present in South Africa between 2012 and 2015, which they believe provides evidence for introduction of clade 2.3.4.4 to South Africa by wild birds.

A new introduction of HPAI (H5N8) virus to South Africa was detected in February 2018, in the northern parts of the country (Abolnik, 2019). Related viruses were found in Nigeria and Poland in July and December 2019 respectively, and Peyrot *et al.* (2022) hypothesise that the South African and Nigerian viruses originated from a common source elsewhere in Africa.

Peyrot *et al.* (2022) sequenced twenty-four HPAI (H5N8) viruses from coastal seabirds in the Western Cape province, sampled between January and May 2018. The seabird viruses apparently originated from a terrestrial bird, southern Cape virus in approximately October 2017 and were the source of viruses that killed at least 500 African Penguins in Namibia in early 2019 (Cannon, 2019; Umberto *et al.*, 2020). Three coastal bird sub-genotypes were observed but no spatial, temporal or species clusters or patterns could be established. Closely-related viruses were widely distributed and at least one seemed to travel almost 200 km within two weeks.

5.2. Clade 2.3.4.4b H5N1 (2020 to 2023)

Clade 2.3.4.4b H5N1 viruses again demonstrated the clade's ability to sweep around the globe, leaving destruction in its wake. These viruses were introduced to West Africa in late 2020 (Lo *et al.*, 2022; Meseko *et al.*, 2023), relatively early in the global panzootic, before many cases occurred in Europe or Asia. Xie *et al.* (2023) found evidence that the African 2021-2022 H5N1 viruses originated in Europe in mid- to late 2020, from H5N8 viruses first detected in Egypt in 2016 and 2017. Significant numbers of H5N1 outbreaks outside Africa only began from October 2021 onwards (Abolnik *et al.*, 2023), leading to another HPAI panzootic that again surpassed previous ones in numbers and species of birds and countries affected (European Food Safety Authority (EFSA) *et al.*, 2023).

Although they had been detected in smaller numbers since the autumn of 2020, detections of HPAI (H5N1) only started to dominate over H5N8 in Europe from October 2021 onwards (EFSA *et al.*, 2023). H5N1 viruses had already become the dominant subtype detected in wild birds, but a novel reassortant was detected in October 2021 (EFSA *et al.*, 2021).

HPAI (H5N1) was first detected in North America on the east coast of Canada in November 2021 and the most closely-related virus was from north-western Europe in spring 2021 (Caliendo *et al.*, 2022). Virus detected in western Canada in February was related to a virus detected in Japan (Alkie *et al.*, 2022).

HPAI (H5N1) reached Central America in October 2022 (EFSA *et al.*, 2023a) and Peru in November, northern Chile by December (EFSA *et al.*, 2023b) and Brazil in May 2023 (Reischak *et al.*, 2023). The most severe conservation impacts in South America, until July 2023, were in Humboldt Penguins and Peruvian Pelicans (*Pelecanus thagus*) (Breed *et al.*, 2023).

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In October 2023 there were reports of HPAI (H5N1) from the Falklands and South Georgia, in the sub-Antarctic, indicating increasing risk to Antarctic wildlife and possibly also to Oceania, including Australia and New Zealand (Banyard *et al.*, 2023).

HPAI (H5N1) outbreaks were reported from Asia in early 2021 and then again from October 2021 onwards, though in smaller numbers than in Europe and the Americas (Abolnik *et al.*, 2023). Though few mortality events were reported, a flock of Whiskered Terns (*Chlidonias hybrida*) died in Taiwan in May 2022 and there was an apparent decrease in Little Terns (*Sternula albifrons*) breeding in Japan in June 2022 (EAAFP, 2022).

5.2.1. Africa

The first outbreaks in wild birds were reported from Senegal and Mauritania in January 2021, involving Great White Pelicans (*Pelecanus onocrotalus*) in Djoudj National Bird Sanctuary and Parc National de Diawling, across the Senegal River. It was estimated that 3000 pelicans were lost, mainly juveniles, comprising 8.4% of the Senegalese colony (Beyit *et al.*, 2023; Lo *et al.*, 2022). In January 2022, Senegal reported another outbreak in Great White Pelican juveniles, of similar magnitude to 2021 (mortality rate 7.7%) (wahis.woah.org, Event 4308). The area hosts overwintering Eurasian ducks, among other Afrotropical and Palearctic migratory birds (Gaidet *et al.*, 2007b; Lo *et al.*, 2022). In addition, viruses recovered from poultry and wild birds in Senegal in 2021 clustered with viruses detected in Europe from October to December 2021 and were hypothesised to have been introduced from southern Europe via migratory birds (Lo *et al.*, 2022).

Nigeria reported its first wild bird cases of clade 2.3.4.4b H5N1 virus in March 2022. These were healthy birds detected inland during a survey in March and April 2022 in the Hadejia-Nguru wetlands, in the northeast (Olawuyi *et al.*, 2024). Twenty-five of 226 birds sampled, originating from three sites, tested positive for clade 2.3.4.4b HPAI H5N1. All species with more than 10 birds sampled at a site had at least one positive, so sample size may have played a role. Positive species comprised White-faced Whistling Duck (9/30 sampled; *Dendrocygna viduata*), Square-tailed Nightjar (3/11; *Caprimulgus fosii*), African Jacana (2/62; *Actophilornis africanus*), Ruff (3/17; *Calidris pugnax*), Spur-winged Goose (2/19; *Plectropterus gambensis*) and White Stork (6/17; *Ciconia ciconia*). The viruses detected did not cluster with those detected in Nigerian poultry since 2021, suggesting a fresh introduction.

A fresh wave of seabird deaths, attributed to HPAI (H5N1) was reported from West Africa from March 2023. Royal and Caspian Terns appear to have been the main species affected, though deaths in Sandwich Terns (*Thalasseus sandvicensis*), Grey-Headed Gulls, cormorants and pelicans were also reported. Senegal, The Gambia, Guinea-Bissau and Guinea reported over 9000 deaths in seabirds (EFSA *et al.*, 2023c, 2023e; Ndao, 2023).

Clade 2.3.4.4b HPAI (H5N1) outbreaks started in South Africa in April 2021 and continued into 2023. This time, the initial cases in seabirds were detected at a similar time to those in domestic poultry, as were outbreaks in different parts of the country. The viruses in southern Africa were linked to those detected in West Africa in from late 2020to early 2021 and were most closely related to viruses detected in Senegal. The southern African viruses were genetically distinct from those detected in Europe and Asia, however (Abolnik *et al.*, 2023).Abolnik *et al.* (2023) sequenced 117 HPAI (H5N1) viruses detected in South Africa between April 2021 and November 2022. Though at least four different sub-lineages were detected in coastal seabirds, mostly gulls, early in the outbreak, a coastal seabird-restricted sub-lineage was detected from September 2021 and persisted throughout 2022, longer than all other South African sub-lineages. These viruses were also, again, the source of outbreaks in Cape Cormorants (*Phalacrocorax capensis*) in Namibia in early 2022 (Molini *et al.*, 2023).

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5.2.2. Israel

A major wild bird mass mortality event that occurred in December 2021 involved the death of at least 10 000 Common Cranes (*Grus grus*) in the Hula valley in Israel and also hundreds of Great White Pelicans. Approximately 40 000 cranes stop over on their way from Russia and Scandinavia to North and East Africa (CMS FAO Co-convened Scientific Task Force on Avian Influenza and Wild Birds, 2022; Lublin *et al.*, 2023; WOAH, 2022). Artificial feeding, causing aggregation of cranes, may have contributed to virus spread and the high mortality (Pearce-Higgins *et al.*, 2023; Pekarik *et al.*, 2022). The cranes were fed to attract them away from agricultural operations such as fish farms. The feeding was halted and carcasses were collected, in an attempt to slow virus spread (Lublin *et al.*, 2023; Pekarik *et al.*, 2022).

5.2.3. Europe

There were very few detections of HPAI in wild birds in Europe between April 2017 and October 2020 (EFSA *et al.*, 2023). This was despite a relatively small peak of approximately 200 detections in poultry in the spring of 2020. After October 2020, the wild bird peaks approximately matched those in poultry, with a period of "peace" through winter 2021, until spring of 2022 when poultry cases declined and wild bird detections rose into another, albeit smaller, peak. They continued at a rate of more than 200 detections a month until August 2023 when there was a brief, two-month, trough before cases rose again in October. HPAI viruses persisted in wild birds through summer, at lower levels in 2021 and higher in 2022, which had not been observed before.

The increase in wild bird detections in October 2020 was due to clade 2.3.4.4b HPAI (H5N8) viruses that dominated HPAI detections until October 2021, when the H5N1 viruses took over. (EFSA *et al.*, 2021).

Waterfowl were the main wild bird category affected until April 2022 and again through the autumn/ winter of 2022/23, but colonial-breeding seabirds became dominant in the summers of 2022 and 2023, during their breeding seasons. Mass mortalities in waterfowl included Barnacle Geese (*Branta leucopsis*) and the deaths of over 20% of the flyway population on the Solway Firth, in Scotland (CMS FAO Co-convened Scientific Task Force on Avian Influenza and Wild Birds, 2022). Species from the Laridae family dominated seabird species detections in general, with a period of dominance by Sulidae (gannets) over August/September of 2022. Among the Laridae species, European Herring Gulls (*Larus argentatus*) were continuously reported as a noticeable proportion of detections, but the Black-headed Gull was noticeably overrepresented from September 2022 until at least July 2023. Common Terns were the second most common source of detections in autumn and winter 2023 and a high mortality rate was observed in juvenile Black-Headed Gulls and Common Terns (EFSA *et al.*, 2023e, 2023d).

The epidemic curve for detections in poultry followed the curve of waterfowl detections rather than seabirds, and poultry outbreaks were believed to be linked more closely to waterfowl. This is consistent with the closer contact between waterfowl and poultry (EFSA *et al.*, 2023a). However, some poultry outbreaks were genetically linked to seabirds in the summer of 2023 (EFSA *et al.*, 2023d).

5.2.4. Great Skuas (Stercorarius skua)

The first mass mortality in European seabirds was in Great Skuas (*Stercorarius skua*), reported a year earlier than the others, in July 2021, from islands off the coast of Scotland (EFSA *et al.*, 2022a). More than 10% mortality was observed at St Kilda and Fair Isle and breeding success was low that season (Banyard *et al.*, 2022).

Great Skuas on the Shetland Isles and St Kilda were again affected in summer 2022, between May and August. Foula (Shetland), with the largest colony globally, had not been confirmed as infected in 2021

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but the mortality rate had been higher than usual and breeding success was poor. In 2022, a 60-70% reduction in nests was estimated. Carcasses were clustered at relatively wet areas where skuas congregate for bathing, which may have also provided a favourable environment for virus transmission. The skuas may initially have been infected by scavenging and (or) predating of other infected species, such as Northern Gannets (*Morus bassanus*) (Camphuysen *et al.*, 2022). However, the risk of virus persisting in the environment until the next breeding season was shown to be low. In October 2022, water samples were taken from under decomposing carcasses and from fresh water sources and no AI virus was detected (Furness *et al.*, 2023).

Preliminary population data available in November 2022 showed that breeding numbers had declined at most monitored sites, since previous counts (Pearce-Higgins *et al.*, 2023).

5.2.5. Dalmatian Pelicans (*Pelecanus* crispus)

One of the earliest HPAI wild bird mass mortality events in 2022 was recorded in Dalmatian Pelicans (*Pelecanus* crispus) in Greece, at the world's largest colony at Prespa Lakes (EFSA *et al.*, 2022b). Mortalities were observed soon after the birds arrived for breeding, in February, and over 1700 carcasses were counted, comprising at least 60% of the colony. A possible source of virus introduction at Prespa Lakes was migratory ducks and resident geese that roost with the pelicans and aggravating factors may have included early breeding combined with low temperatures and high pelican density. It seems that all carcass removal was done as one operation and was found to be challenging, along with the monitoring and coordination of stakeholders. Another three Greek colonies, in the east, were affected, as well as colonies in Albania, Montenegro and Romania. Deaths equated to at least 40% of the south-eastern European population and 10% of the global population (Alexandrou *et al.*, 2022).

The outbreak in Montenegro, at Skadar Lake involved a much smaller colony, but 10-12% of the population was lost and breeding failed completely. This was due to both human disturbance before the outbreak and the outbreak itself (Vizi, 2022).

5.2.6. Sandwich Terns (Thalasseus sandvicensis)

Knief *et al.* (2024) estimate that at least 17% of breeding Sandwich Terns from north-western Europe may have died from HPAI in May and June 2022 and most chicks died in breeding colonies, even where few adults died. Colonies appeared more likely to be affected if they were larger and closer to the centre of the breeding range. It was postulated that adults moving between colonies may have spread the virus but found that there were at least three separate introductions to the population, possibly via geese and gulls (Rijks *et al.*, 2022). Mortality rates slowed over time, possibly due to less favourable environmental conditions later in the season, decreased density in colonies and/ or seroconversion and improved herd immunity. There was also an association between carcass removal and reduced outbreak severity. Live birds were seen interacting with dead ones, which may partially explain this. Distance to water, vegetation density and soil permeability were not associated with outbreak severity (Knief *et al.*, 2024).

Overall, nine of ten of the colonies in the Netherlands were affected (Rijks *et al.*, 2022) and the mortality rate was estimated as 17.2%–90% of the summer migrant population and more than 1000 times higher than the summer average from previous years (Caliendo *et al.*, 2024). Affected birds were debilitated and lethargic and "some" displayed apparent neurological signs such as opisthotonus and "flipped over backwards". Histopathology and immunohistochemistry were performed on organ samples from four adults and two chicks. Immunohistochemistry in the adults' samples detected virus most often in the duodenum and pancreas, which was associated with necrosis and inflammation. The death of the chicks was attributed more to starvation, probably because the parents died (Rijks *et al.*, 2022).

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A range in mortality rates was estimated from the German Wadden Sea but over 60% mortality was estimated in one Sandwich Tern colony (Pohlmann *et al.*, 2023). Two genotypes were involved with tern and gannet outbreaks, already detected in Germany.

5.2.7. Northern Gannets (*Morus bassanus*)

The first cases of HPAI H5N1 in Northern Gannets were detected in Iceland in April 2022 and outbreaks continued until September, with high mortality reported at 40/53 colonies (one of the remaining 13 colonies was not affected and 12 were not monitored). North American gannets were affected from May, at a similar time to those in the east Atlantic, and continued through June (Lane *et al.*, 2023). Over 50% of Northern Gannets may have died (Grémillet *et al.*, 2023), with a 71% reduction in occupied nest sites at Bass Rock in Scotland, the largest Northern Gannet colony, estimated using drone footage (Lane *et al.*, 2023). This could have equated to at least 3% of the overall breeding population, though the outbreak seemed to be over by the end of July (Lane *et al.*, 2023). It appeared that colonies affected earlier in the breeding season had more severe outbreaks (Camphuysen *et al.*, 2022; Knief *et al.*, 2024; Lane *et al.*, 2023).

Great Skuas may have introduced the virus and gulls and (or) immature gannets may have spread it between colonies. Another theory is that the gannets may have behaved abnormally during the outbreak and spread the virus themselves (Lane *et al.*, 2023). Breeding gannets usually would not visit other colonies or make contact with birds from other colonies during foraging. However, Jeglinski *et al.* (2024) observed highly unusual movements by 3 of 10 adult gannets from Bass Rock, tracked before and during the outbreak. In the middle of the outbreak, after all tracked birds had lost their eggs or chicks, two visited other colonies and, slightly later, one visited a freshwater lake far inland. This behaviour had not been recorded before, even with failed breeding attempts. An added factor may be a mate not returning, which could have occurred here. However, this is not discussed. Both of these birds survived until at least September and one had a black iris, but the tag of the third bird stopped transmitting soon after the trip inland and the bird was not re-sighted. Serology from samples taken on Bass Rock in September also provided evidence of infected birds that had survived and birds with dark irises were more likely to be seropositive (Lane *et al.*, 2023).

The unusual behaviour recorded by Jeglinski *et al.* (2024) was not observed by Grémillet *et al.* (2023) when they tracked gannets from three large colonies (Bass Rock, Grassholme in Wales and Rouzic in Brittany, France) after the HPAI outbreaks, during the late chick-rearing period. The only difference noticed, when the data were compared to that from the early chick-rearing phase in 2019 (before any HPAI outbreaks), was that foraging effort seemed lower. This could be due to reduced competition after the outbreak and reduction in breeding numbers but just may be due to the different chick-rearing phases studied.

Besides reporting on laboratory confirmation of HPAIV H5N1 presence at each colony, Grémillet *et al.* (2023) also report on additional testing of juveniles at Rouzic, sampled between July and September 2022. Though all seven birds tested negative on cloacal and tracheal swabs, virus was detected on external wipes (feathered and unfeathered areas of the body) and four birds were seropositive.

The mortality rate in non-breeding gannets in the Netherlands was estimated at 33%–90% and was 66 times higher than the summer average from previous years (Caliendo *et al.*, 2024).

5.2.8. Common Terns (*Sterna hirundo*)

In June 2022, HPAI H5N1 was detected in a mixed colony of Common and Sandwich Terns breeding on the German Wadden Sea coast. Two of the German Wadden sea colonies experienced at least 37 and 46% mortality respectively, though the third colony was more mildly affected (0.3% estimated). This was the largest colony but the nest density was relatively low, which may have slowed virus spread (Pohlmann *et al.*, 2023).

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5.2.9. North America

The first HPAI H5 detection in North America, since 2015, was in a Great Black-backed Gull (*Larus marinus*) on the east coast of Canada in November 2021 (Caliendo *et al.*, 2022). The virus was possibly transferred from Europe to North America via Iceland (Gass *et al.*, 2023). By the end of December, HPAI H5N1 viruses had been detected in the USA, in hunter-harvested ducks sampled as part of routine official surveillance in South Carolina. Another 291 wild bird detections from 13 states on the US Atlantic Flyway had been made by the beginning of March 2022, all from apparently-healthy dabbling ducks (Bevins *et al.*, 2022). In February 2022, H5N1 was detected in a Bald Eagle (*Haliaeetus leucocephalus*) in British Columbia, western Canada (Alkie *et al.*, 2022). The first detection in the west of the USA was in Alaska in April 2022 and the viruses detected were related to those from Japan and Canada (Youk *et al.*, 2023).

There was evidence of another virus introduction to Canada from Europe in the winter of 2022/23, found in a Red Fox (*Vulpes vulpes*) and American Crow (*Corvus brachyrhynchos*) from Prince Edward Island (Alkie *et al.*, 2023).

By March 2023, there had been an estimated minimum of 31 mass mortality events in wild birds in the USA, reported via federal agricultural and environmental agencies, with 100 to 3755 birds affected in each event. Seabird species involved in mass mortality events in North America included the American White Pelican (*Pelecanus erythrorhynchos*), Caspian Tern, Double-crested Cormorant (*Nannopterum auritum*) and Northern Gannet (Harvey *et al.*, 2023).

Cormorants in North America were affected by H5N1 in the spring of 2022 and Caspian Terns in autumn, with die-offs of Common Murres and Northern Gannets in Canada (EFSA *et al.*, 2023a). In spring 2023, there were fewer wild birds affected in North America, but a significant proportion (21/561= 4%) of California Condors (*Gymnogyps californianus*) were lost to HPAI (EFSA *et al.*, 2023c).

Giacinti *et al.* (2023) described the results of avian influenza surveillance efforts in Canada from November 2021 to November 2022. Of approximately 6200 sick and dead wild birds, 27.4% tested positive and 5.2% of approximately 11 200 apparently healthy birds, providing evidence of potential reservoirs. Most virus identified in the Atlantic flyway was Eurasian HPAIV, though only 25% of viruses overall were of Eurasian origin and the rest were reassortants between Eurasian and North American viruses. Reassorted viruses dominated those detected in healthy birds. Index cases detected in the three flyways were usually Anseriforms, some of them healthy, or clinically-affected raptors or corvids but subsequent cases were predominantly sick and dead Charadriiforms, mostly gulls. Mass mortalities occurred from March 2022 onwards, mostly in Anseriforms and raptors in earlier months but seabirds in eastern Canada predominated in spring and summer.

Mass mortalities were observed in eastern Canada from spring 2022 and, between April and September 2022, and wild bird deaths exceeded 40 000 in eastern Canada, dominated by seabirds and sea ducks. This included at least 25 000 Northern Gannets, 8 000 Common Murre, 1 900 American Common Eider (*Somateria mollissima dresseri*), 2 300 gulls, 900 cormorants, and smaller numbers of Atlantic Puffin (*Fratercula arctica*), Black-legged Kittiwake (*Rissa tridactyla*), Razorbill (*Alcatorda*), and terns (Avery-Gomm *et al.*, 2024). Mass mortalities elsewhere included Double-crested Cormorant (*Nannopterum auritum*) and American White Pelican (*Pelecanus erythrorhynchos*) (Giacinti *et al.*, 2023). The virus was expected to have had an effect at population level in the gannets (at least 11% of North American Population) and eiders (12% loss of breeding females), but “apparently occupied territories” in the gannet colony decreased by 58% on one island in 2022 (Avery-Gomm *et al.*, 2024). Monitoring methods included aerial surveys, which showed evidence that the first report of mass gannet mortality in Newfoundland missed the start of the outbreak by a few weeks. There were no reports of mass mortality on the same scale, during the same period, from the eastern United States.

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A colony of American Herring Gulls (*Larus argentatus*) in New Brunswick, Canada, were closely studied through summer 2022. Less than 10% of breeding adult mortality was attributed to HPAI, though mild clinical signs were more widespread. Neurological clinical signs dominated, including lethargy and drooped wings and progressing, in more severe cases, to signs such as torticollis. The duration of clinical signs before death was estimated at a maximum of 36 hours and R_0 was estimated at 8.23 while the incidence was increasing, 0.84 while the incidence was decreasing and 1.02 overall. The colony was less than a quarter of the size of previous years, which may have contributed to lower density and slower virus transmission. Great Black-backed Gulls, two anatid species and crows breeding on the island were also suspected or confirmed to be affected by HPAI. Though apparently not affected, seals were seen consuming gull chicks in the water (Taylor *et al.*, 2023).

5.2.10. Central and South America

HPAI H5 virus was detected in Colombia in October 2022 and between December and January in Central America, where it was associated with the deaths of hundreds of Brown Pelicans (*Pelecanus occidentalis*) (Breed *et al.*, 2023).

In Peru, the virus was detected in dead Peruvian Pelicans in mid-November, where at least 200 died (EFSA *et al.*, 2023b). By mid-March 2023 at least 220 000 birds of at least 24 species were believed to have been affected in Peru, with sick birds showing neurological signs. In protected areas, the most numerous carcasses were of Peruvian boobies (*Sula variegate*; 47 500), though this equates to a maximum of 4.8% of the Peruvian population. Affected Guanay cormorants (*Leucocarbo bougainvilliorum*) were estimated at 29 000 (1.4%) and also more than 21 000 Peruvian pelicans. The latter, however, accounts for between 13 and 25% of the Peruvian population and up to 21% of the global population, with proportionally the largest effect (Gamarra-Toledo *et al.*, 2023). By July 2023, the Peruvian government had reported over half a million deaths in seabirds (Breed *et al.*, 2023).

South American sea lions (*Otaria flavescens*) in Peru were experiencing mass mortalities attributed to HPAI by early 2023. Sequencing of viruses from a common dolphin (*Delphinus delphis*), three South American sea lions, four pelicans, a sanderling and a Guanay Cormorant showed that the viruses were reassortants, with half the gene segments clustering with Eurasian H5N1 viruses and half with North American lineage viruses. Viruses with the same reassortment pattern had been detected in Colombia and Ecuador in November 2022 and in North America since March 2022, becoming dominant thereafter (Leguia *et al.*, 2023).

Guano is still harvested from seabird colonies in South America and HPAI therefore also poses a measurable economic threat to this industry. The further study of bird movements and migrations, in relation to HPAI spread, was recommended, given seabirds and other species that are known to move long distances in South America, between countries (Gamarra-Toledo *et al.*, 2023).

HPAI (H5N1) was detected in Chile in December 2022 in Peruvian Pelicans (*Pelecanus thagus*) and the viruses were linked to those detected in Peru, Ecuador and Mexico, and to a progenitor in North America (Ariyama *et al.*, 2023; Jimenez-Bluhm *et al.*, 2023). A surveillance program in place since 2015 detected an increase in AIV detections in November and December 2022, from less than 1% to 2.6 and 11.7% respectively, and 7/69 positive environmental faecal samples (10%) contained H5 viruses (Jimenez-Bluhm *et al.*, 2023). The monthly maximum AIV-positive sample proportion, from 2015 to 2020, had been 8.6% (Ruiz *et al.*, 2023).

By the beginning of March 2023, Chile had reported a total of 197 laboratory-confirmed H5N1 outbreaks to WOAH, including 72 in Peruvian Pelicans, 26 in Peruvian Boobies, 20 in Kelp Gulls (*Larus dominicanus*) and two in Humboldt Penguins (EFSA *et al.*, 2023). Soon afterwards, Chile reported mortalities in an estimated 10% of their Humboldt Penguin population, with four confirmed cases (Leguia *et al.*, 2023). By May 2023, the Chilean government had reported approximately 28 000 deaths

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in seabirds, including boobies, cormorants, pelicans and nearly 5000 Kelp Gulls. By July they had reported over 460 Magellanic Penguins (Breed *et al.*, 2023) and a media report stated that up to 3000 Humboldt Penguins had died of HPAI by October 2023. As Chile is estimated to have approximately 5100 breeding pairs and the global population is about 24 000 individuals (datazone.birdlife.org), this is a significant proportion of the population (EFSA *et al.*, 2023).

Ruiz *et al.* (2023) linked increased AIV detection risk in Chile to higher vegetation coverage and abundance of migratory birds and isolated ten AIV from six species: gulls, oystercatchers, a plover and a Chilean Flamingo (*Phoenicopterus chilensis*). The plover and Franklin's Gull are migratory and the others, except the flamingo are resident. The flamingo was classified as "accidental".

The first HPAI (H5N1) detection in Argentina was in February 2023 (Breed *et al.*, 2023) and more than 60% of a tern colony died in Patagonia in October and November 2023. Though the virus was also detected in Kelp Gulls, they appeared more resistant, with lower mortality and milder clinical signs (Banyard *et al.*, 2023).

The first detection of HPAI (H5N1) in Brazil was in May 2023, in migratory Cabot's Terns (*Thalasseus acuflavidus*), as well as in other wild species and backyard chickens. Phylogenetic analysis linked the viruses from the terns to those detected earlier in Peru and Chile (Reischak *et al.*, 2023). Mortality in seabirds remained relatively low, except for deaths in over 900 terns of different species between May and July, though there were high mortalities in marine mammals in October (Banyard *et al.*, 2023; Breed *et al.*, 2023).

HPAI (H5N1) was detected in the Falkland Islands in October 2023 in Southern Fulmars (*Fulmarus glacialoides*) and Black-browed Albatrosses (*Thalassarche melanophris*) (Banyard *et al.*, 2023; Bennison *et al.*, 2023). At least 30 albatrosses died and there are concerns for the 200 000 pairs breeding on the island, but no reports of mass mortality seem available as of 30 January 2024.

5.2.11. Sub-Antarctic region

At the same time, HPAI (H5N1) was sampled from Brown Skuas (*Stercorarius antarcticus*) and Kelp Gulls on South Georgia, in the sub-Antarctic, and mass mortalities in southern elephant seals (*Mirounga leonina*) were observed (Banyard *et al.*, 2023). Infection was later confirmed in the elephant seals, as well as in fur seals and Antarctic Terns (British Antarctic Survey, 2024).

6. Avian influenza surveillance in wild birds

6.1. Introduction

Wild bird surveillance for avian influenza viruses is necessary both as an early warning system and as a tool to understand the disease's epidemiology. Early detection of novel high pathogenicity viruses or, in the Northern Hemisphere, viruses introduced during autumn migration, allows better preparation for outbreaks. Understanding which species are involved in virus introduction, maintenance and transmission is also necessary for effective disease mitigation (Verhagen *et al.*, 2021).

Wild bird samples for AIV testing can be obtained most easily from dead birds (that were hunted, euthanised or succumbed to the virus) or environmental samples and, with more effort, from live birds that are caught or trapped, either explicitly for AIV sampling or for other purposes such as tagging, rehabilitation or research (Waldenström *et al.*, 2022). Wild bird surveillance programs can be labour-intensive and expensive, requiring access to difficult terrain, expertise in effective capture methods and funding for transport, consumables and costly virus-detection assays and genomic sequencing.

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The value of the programs may also be difficult to defend between panzootics (Machalaba *et al.*, 2015) or when there are limited resources for disease mitigation or limited resources in general.

Collection of environmental samples (faeces, water, mud) is the most convenient option and can be very informative about what viruses are circulating. However, information about host species, age and other individual information may be lost. Species can be determined by taking the effort to watch birds defaecate, and mitochondrial DNA analysis of faecal samples can determine species, but this will add complexity and expense. Some HPAI strains may also not be present in faeces at detectable levels so environmental sampling may be a less sensitive early warning system for HPAI virus introductions (Hood *et al.*, 2021). Sampling dead birds can also be relatively convenient but may miss low pathogenicity viruses or species that are less susceptible to a HPAI virus. Encouraging the public to report sick and dead birds, and patrolling more remote areas can help to ensure that disease is detected as early as possible (Waldenström *et al.*, 2022). Clear guidelines on reporting routes and effective communication systems are important to ensure that observed sick or dead birds can be sampled or accurately counted. The World Organisation for Animal Health's (WOAH) Terrestrial Animal Health Code advises that targeted active surveillance may be necessary in some species to detect subclinical infections and to improve understanding of HPAI epidemiology. There should be a focus on high-risk periods, species and areas (World Organisation for Animal Health (WOAH), 2023). Examples of surveillance systems in the Northern Hemisphere are provided below.

6.2. Avian Influenza surveillance in wild birds in Africa

Detection of HPAI in wild birds in Africa is generally based on testing of sick and dead birds, but government funding for laboratory testing is scarce. Pavade *et al.* (2010) conducted a survey via OFFLU and found that wild bird surveillance was only reported from South Africa and Zimbabwe. The number of countries doing surveillance is not expected to have changed much since then. Few scientific manuscripts have described avian influenza surveys since 2020 and these seem to have been funded externally. Surveys involving collection of wild bird faeces in Zambia and Kenya were funded by a World Bank funded project on infectious diseases and by sources in Japan and by U.S. Centers for Disease Control and Prevention under the research cooperative agreement with Washington State University (Kalonda *et al.*, 2023; Munyua *et al.*, 2024). The Food and Agriculture Organisation of The United Nations (FAO) Emergency Centre for Transboundary Animal Disease Control regional offices in Eastern and Southern Africa fund laboratory consumables in Ethiopia, Kenya, the United Republic of Tanzania and Uganda through a USAID funded Global Health Security programme (FAO Emergency Prevention System for Animal Health (EMPRES-AH), 2024). These projects have limited lifespans by nature, so a more sustainable model is needed to build and maintain effective HPAI surveillance in Africa.

Wild bird avian influenza detection in South Africa is currently mainly funded through the University of Pretoria, by a National Department of Science and Innovation (DSI) and National Research Foundation (NRF) grant and National Department of Trade and Industry (DTI) grants, with contributions from provincial governments and poultry companies (Abolnik *et al.*, 2024, 2023). Seabird testing has been funded through a seabird rehabilitation centre that applied for project and emergency outbreak funding and through the University of Pretoria, through funding for the PhD work described in this dissertation. Again, a more sustainable long-term surveillance program is needed. There are no guidelines provided by government and government funded testing is largely opportunistic and in response to disease events.

6.3. Wild bird avian influenza surveillance in the Northern Hemisphere

Testing of sick, dead and moribund wild birds is prescribed by the European Union (EU) (The European Commission, 2020). A list of target species is provided, which includes ducks, geese, birds of prey and several other water bird species. Brouwer *et al.* (2018) described a recommended reporting system

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for avian influenza surveillance in the EU. They requested that the population numbers of the target species be supplied by member states where possible so that sampling effort can be estimated. They also provide suggestions for accurate identification of wild bird species, from sending photographs to ornithologists, using Cornell Laboratory of Ornithology's Merlin Bird ID App, providing a smartphone application that allows people to send photographs of dead and moribund birds to the veterinary authorities (like the Danish Veterinary and Food Administration (DVFA) with their FugleinfluenzaTip ('Bird fluTip')) to DNA bar- coding. They reported challenges with receiving accurate geographic locations and recommended that pathological examinations of sampled dead birds be performed to estimate the pathogenicity of the virus in that species. The age of sampled birds was also deemed important.

In the United States, wild migratory birds are actively sampled along the flyways according to the "Surveillance Plan for High pathogenicity Avian Influenza in Wild Migratory Birds in the United States" (2017). This plan used data from a previous avian influenza surveillance effort in the USA that ran for five years from April 2006 to the end of March 2011and involved collection of nearly two hundred thousand samples. The previous program targeted bird species known to migrate directly from Europe and Asia, birds in contact with species from areas with reported outbreaks and known reservoir species. It was able to detect geographic hotspots of AIV in the United States and identify patterns of infection in terms of species affected and seasonal occurrence (Bevins *et al.*, 2014).

6.4. Conclusion

A surveillance program of the same scale as the United States' is not currently feasible in Africa, but would be ideal. Sampling of scientifically-identified high-risk sites, starting with environmental faecal samples, would provide valuable information on circulating viruses. The program described by the EU is closer to the current monitoring in place, but effectiveness would be improved by better communication with the public and clear reporting guidelines and channels. Funding for testing, and probably laboratory capacity, are still major hurdles, especially between outbreaks.

7. HPAI management in wild birds

Since 2021, annual statements have been published by a Scientific Task Force on Avian Influenza and Wild Birds, co-convened by the Convention on the Conservation of Migratory Species of Wild Animals (CMS) and the Food and Agriculture Organization of the United Nations (FAO). The statements have included an update on the global situation and issues of conservation concern, as well as recommendations for preparation for and response to outbreaks, based on the latest scientific literature. This advice is supported by resolutions made by multilateral environmental agreements such as the CMS, Agreement on the Conservation of African- Eurasian Migratory Waterbirds (AEWA), the Ramsar Convention on Wetlands and the Convention on Biological Diversity (CBD).

The Task Force advises that governments take responsibility for HPAI planning, given the biodiversity threat, and that there is inter-sectoral collaboration. This should include an interdisciplinary expert panel. Both risk mitigation (including surveillance) and emergency response plans should be drafted and resources provided to implement them. The plans should include information to aid collaboration, including descriptions of roles and responsibilities and communication pathways, and should include guidance on how to prevent human exposure to virus.

Other conservation activities should be intensified to try to reduce the overall pressure on affected/ at-risk populations and site management plans should include contingency measures. This should include risk assessment, comprising an evaluation of population vulnerability and the potential impact of an HPAI incursion. There is acknowledgement of knowledge gaps that hinder risk assessment, but

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frequent contacts between birds nesting in colonies could increase vulnerability and impacts will be higher in long-lived birds that breed at an advanced age.

Further advice on contingency planning includes biosecurity measures, monitoring for early detection of an outbreak and obtaining data on its development and impact, making decisions about carcass removal, safeguarding human health, communicating with the public and the need to reform poultry systems to prevent future panzootics.

Possible harmful effects of carcass removal include disturbance of breeding, additional virus spread through displaced birds, people, vehicles and carcasses, and increased human health risk. Important considerations include availability of resources, site accessibility, number of carcasses and feasible disposal options (CMS FAO Co-convened Scientific Task Force on Avian Influenza and Wild Birds, 2023).

Furness *et al.* (2023) could not detect AIV RNA in water from under rotted skua carcasses, four months after an outbreak at Foula, Shetland. They conclude that carcass removal may not therefore affect the probability of an outbreak in the next season, but suggest that removal of fresh carcasses, early in an outbreak, may still be beneficial. They also discuss differing scavenging behaviour between small and large skua colonies (more scavenging in smaller colonies) and they hypothesise that mortality rates should be higher in smaller colonies if scavenging is an important route of infection.

The Scottish Government requested an assessment of the possible effects of carcass removal in seabird colonies, by the Centre of Expertise on Animal Disease Outbreaks (EPIC). The resulting report of a qualitative risk analysis concluded that carcass removal was unlikely to cause a significant reduction in virus transmission. However, there was high uncertainty linked to the likelihood of infection from a carcass, the relative contributions to environmental contamination of carcasses and live infected birds, and the relative roles played by direct (from live infected birds) and indirect (via environmental contamination) transmission (Adam *et al.*, 2023).

Kleyheeg *et al.* (2020) discussed the wild bird HPAI outbreaks in the Netherlands in 2016-2017 and recommend monitoring sites where birds arrive after migration, increasing awareness of clinical signs to detect infections as early as possible, detailed surveillance during outbreaks, specific guidelines for handling carcasses and reporting, and that resources should be available to allow for effective sampling and monitoring.

An “AI-Impact working group” was established in the Netherlands in 2020; a multidisciplinary group to monitor the effects of HPAI in wild birds. Species, dates and locations of dead birds were reported to this group by government officials and by members of the public via different web applications owned by member organisations of the working group (Caliendo *et al.*, 2024). Beached bird survey data were included (Camphuysen, 2022) and mortality rates were compared with previous years as a baseline. Carcasses were cleared to lower the level of contamination in the environment but advice was provided on how important this may be in each specific situation (Caliendo *et al.*, 2024).

Pohlmann *et al.* (2023) described the HPAI outbreaks in seabirds in summer 2022, in the German Wadden Sea. Tern breeding colonies were not entered in Schleswig-Holstein and Lower Saxony, to avoid disturbance and possible further virus spread. At one colony, dead birds were counted daily along a 1.3 km transect near the colony and at another, carcasses were counted at the end of the breeding season. At other sites, all carcasses were removed twice a day, and some were counted and some not. At the gannet breeding site on Heligoland, carcasses were collected on three dates and regular checks were made at designated areas of the coast. Knief *et al.* (2024), presumably incorporating the Dutch data from Rijks *et al.* (2022), found an association between Sandwich Tern carcass removal and reduced outbreak severity in north-western Europe.

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Meier *et al.* (2017) reported on the outbreaks of H5N8 in wild birds in Switzerland at the end of 2016. Only a few carcasses from clusters of deaths were tested, and sites previously positive were not re-tested. This meant that laboratories were not overwhelmed but there was the disadvantage that the exact numbers affected were unknown. This may not be possible to remedy by testing in future, if outbreaks occur on a larger scale and (or) laboratory capacity is not sufficient. Management actions commended during stakeholder discussion included rapid decisions made by the veterinary authority in consultation with stakeholders, and good communication with the media and via regular updates on their website. Recommendations for the future included better distribution of materials for sampling and better sampling instructions, e.g. to take cloacal and choanal swabs and not to transport carcasses, as this increases the risk of disease spread.

8. Vaccination against avian influenza

8.1. Planning a vaccination program for wildlife

8.1.1. Objectives and associated vaccine specifications

Effective vaccination of any population requires careful planning and preparation. However, compared to domestic animals, wildlife vaccination involves less controlled and more variable populations and environments and therefore more unpredictable disease and response to vaccination (Barnett and Civitello, 2020). Preparation therefore becomes even more important.

Common aims of wildlife vaccination include the conservation of vulnerable species and (or) protection of other species from pathogen spill-over. These aims dictate whether pathogen eradication is required and therefore how effective the vaccine needs to be (Barnett and Civitello, 2020). Aiming only to prevent disease with a vaccine may be justified in endangered species when concerned with protecting individuals, but vaccines that prevent transmission are generally more efficient and cost effective (Wobeser, 2002).

The aims of a vaccination program should be defined clearly from the start and aligned with the capabilities of vaccine candidates. Few vaccines prevent all infections, and the consequent disease and pathogen shedding. Some induce waning, binary or partial immunity. Immunity may not last or may need repeated vaccinations to maintain sufficient levels (waning), and some individuals may never develop immunity while others react fully (binary immunity). A vaccine may achieve only partial immunity by failing to prevent infection or morbidity and mortality and (or) allowing shedding and further pathogen spread. Besides not achieving its purpose, partial immunity could select for increased pathogen virulence. Vaccines that convey only partial protection against infection and shedding may raise the infectious dose required and drive the evolution of a raised pathogen reproduction rate. Animals that are protected from death, but are not prevented from shedding, may survive to spread virulent pathogen for longer (Barnett and Civitello, 2020).

One of the few, if not the only, example of wild bird avian influenza vaccination is that of vaccination of California Condors (*Gymnogyps californianus*). The United States Department of Agriculture's Animal and Plant Health Inspection Service approved vaccination of critically endangered Californian Condors after twenty-one out of 561 died from HPAI in March and April 2023 (Bartels, 2023). Approval was granted on the grounds that they are critically endangered, carefully managed and the small population allows monitoring and control of the vaccine. An inactivated vaccine was approved and trials in black vultures, as a less vulnerable species and relatively close relative of condors, were carried out to assess the safety and immunogenicity of the vaccine and the required dosing regimen. Since safety and satisfactory antibody levels were established, trials in a small number of captive condors started in July 2023. However, two doses were required to achieve antibody levels consistent with protection in 9/10 vultures and only 7/10 of the single-dose group had protective antibody levels (U.S. Fish and Wildlife Service, 2023). In such an endangered population and with probable outside sources

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of HPAI virus, 70% protection is probably unacceptable and even 90% may be too low. However, the feasibility of this level of coverage would need to be assesses and weighed up and the risk that the vaccine will not be completely effective needs to be weighed against the resources required to carry out the vaccine program and the associated risks to the condors (injury, disrupted breeding etc.).

8.1.2. Disease epidemiology and population ecology

Vital to vaccine program planning, and intertwined with the minimum vaccine standards is the epidemiology of the disease and the population characteristics of the target species. Maichak *et al.* (2017) assessed three decades of vaccination of wild elk in Wyoming, USA, against *Brucella abortus.* They concluded that, although an estimated average of 97% of juvenile females had been vaccinated each winter, at feeding grounds, vaccination had not reduced *B. abortus* seroprevalence and outbreaks in cattle had not declined. Vaccination was achieved through the use of innovative freeze-dried Strain 19 in methylcellulose ballistic "biobullets" (Herriges *et al.*, 1992), which had been determined to be almost as effective as hand-injected strain 19 vaccine. However, the biobullets had been shown to only reduce abortions by 25%, to 33%, in infected cows. This was presumably thought to be enough of an effect to reduce disease spread and justify the effort and expense of the vaccination program. However, it seems that the decision was taken without enough information on the epidemiology of the disease and without considering the need for other supporting interventions. Maichak *et al.* recommend further investigation into oral vaccines, that could be more efficient and cost-effective, and further use of other management strategies such as dispersal of feeding sites and shortening of feeding periods, as well as reducing interference with scavengers, which remove aborted material.

Another example of insufficient epidemiological information was recorded in New Zealand. In 2004, almost the entire critically endangered kakapo (*Strigops habroptilus)* population of 124 were vaccinated for *Erysipelothrix rhusiopathiae* after three juveniles died from erysipelas after translocation. Kakapo are a parrot endemic to New Zealand and are extinct in their natural range, with three managed populations maintained on offshore islands. *E. rhusiopathiae* was found in seabird carcasses inland where the juveniles originated and the decision was taken to vaccinate. However, after the development of a suitable ELISA, it was found that most birds had had relatively high antibody titres pre-vaccination and that vaccination did not raise the average population antibody levels (Gartrell *et al.*, 2005; Livingston *et al.*, 2013). This is an example of where vaccination was feasible, and seemed justified when considering a possible threat to a tiny population. However, knowledge of the disease's epidemiology was lacking and, in the end, any benefit may have been outweighed by risks.

In a closed population, after a certain proportion of the population is immunised, herd immunity starts to have an effect in protecting the remainder of the population and 100% vaccination coverage may not be necessary. The reproductive rate of a disease (R_0), related to population density, infectiousness of the agent and proportion susceptible, dictates how much of a population must be vaccinated to halt the spread of the disease. The turnover rate of the population will affect the proportion susceptible and therefore also the vaccinated proportion required (Wobeser, 2002). If the virus is constantly introduced from other species, it is possible that up to 100% immunisation may be needed to prevent deaths and disease. Mathematical models may help to explore some of these factors and aid in effective planning (Bourret *et al.*, 2018; Cleaveland, 2009). However, the more epidemiological and population ecology data that can be fed into these models, the more helpful they will be.

8.2. Influenza vaccine-related immunology

Defences against pathogens in mammals and birds can be classified as being part of the innate or adaptive immune system. Infection by influenza viruses is primarily through respiratory or digestive mucosa (Swayne and Kapczynski, 2008). Innate mucosal immunity includes physical defences such as

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cilia, mucus and the epithelial barrier and chemical defences such as complement molecules and antimicrobial peptides. After the virus has penetrated the epithelium, there is a response from immune cells such as neutrophils, heterophils and macrophages, which both attack the virus and release signal molecules, such as cytokines and chemokines, which activate cells (lymphocytes) comprising the adaptive immune system. Phagocytic antigen-presenting cells (APCs) transport viral proteins to the local lymphoid tissue and display the antigen to naïve T lymphocytes. There is selection for the cells with the correct randomly-generated specific antigen receptors, and these are recruited to the site of infection where cytotoxic (CD8) T cells attack the virus. Production of interferon by APCs and T helper (CD4) cells activate antibody (immunoglobulin)-secreting cells, i.e., B lymphocytes, to produce secretory immunoglobulin (Ig) A in the *lamina propria*, which binds virus on the mucosal surface, and IgG, which seems to play an important role in the lower respiratory tract of mice (Calzas and Chevalier, 2019).

Antibodies provide what is termed humoral immunity. By binding virus, antibodies play a role both in tagging it for destruction by cells, and by preventing it from binding to and infecting cells. Once the infection is cleared, memory T and B cells will develop and remain in lymphoid tissue, bone marrow and circulation. The next time a similar virus infects the body, they will launch a more prompt and greatly magnified response, (Calzas and Chevalier, 2019; Roitt *et al.*, 2002; Swayne and Kapczynski, 2008).

The influenza A antigen sites to which T cells bind (epitopes), e.g., on the nucleoprotein, matrix protein 1 and on surface proteins, are common across influenza A viruses (IAV). Therefore, previous exposure to an IAV may provide partial protection against a new infection with a relatively unrelated virus (Calzas and Chevalier, 2019). Exploitation of these conserved proteins may also be the answer to a universal influenza A vaccine (Ninyio *et al.*, 2020). Secretory IgA is similarly non-specific but the majority of anti-IAV antibodies are specific for the haemagglutinin 1 protein subunit, which is highly variable, and therefore they provide protection against a narrow range of viruses (Calzas and Chevalier, 2019).

8.3. Avian influenza vaccines

Swayne and Sims (2021) list the properties of an ideal vaccine for poultry, which include: 1) low cost, 2) effective in multiple species and (3) effective after a single dose, 4) easily applied to large numbers of birds, 5) ability to distinguish infected from vaccinated birds (DIVA), 6) effective in young birds (ideally day- olds) with residual maternal antibodies and 7) close antigenic match to field virus.

Leslie Sims has compiled a list of twelve aspects of good “Vaccine Stewardship”, which were explained by David Swayne at an FAO meeting in April 2023 (Swayne and Sims, 2023) and by Sims at the Global Consultation on High Pathogenicity Avian Influenza (Food and Agriculture Organisation of the United

Nations (FAO), 2023). The points relevant to wild birds include employing other mitigation measures where possible and not just relying on vaccination, using a vaccine that protects against circulating strains, and at the correct dose and time, and monitoring vaccinated birds to ensure immunity is achieved and maintained (with boosters if necessary). Circulating viruses need to be monitored for antigenic changes or new introductions that may require updating of vaccines. It should also be ensured that the teams performing vaccination do not contribute to virus spread and the vaccination program must be regularly re-assessed to gauge its ongoing necessity and effectiveness.

Avian influenza viruses evolve rapidly through genetic mutation and reassortment of gene segments and vaccines against high pathogenicity strains can lose effectiveness fast. Vaccine seed strains must constantly be compared to circulating field viruses, and updated if necessary, to ensure continued protection and prevent extra evolutionary pressure. Vaccination may have been one cause of the rapid evolution, associated with antigenic variation, of the Goose-Guangdong lineage viruses that emerged

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in the 1990s (Lewis *et al.*, 2021). Cattoli *et al.* (2011) found higher evolutionary rates, and numbers of codons that were likely under positive selective pressure in viruses, from countries that vaccinated against HPAI H5N1, relative to those that did not. These countries had carried out unsuccessful vaccination programs, though other countries' programs had been effective. Possible causes for failure were poor vaccine coverage and poor vaccination monitoring. Vaccination against a LPAI H6N2 has been done in South Africa since 2001, with a vaccine derived from one of the two original sub-lineages. Viruses of both lineages, detected in 2012 and 2013, were compared with viruses from 2002 and there was a much higher mutation rate in the vaccine strain sub-lineage (Rauff *et al.*, 2016).

Commercially available vaccines include 1) inactivated, adjuvanted whole virus vaccines, 2) recombinant vaccines with HA and (or) neuraminidase (NA) genes inserted into live virus vectors, such as Herpes and Newcastle disease viruses, 3) haemagglutinin DNA administered in a plasmid (AgriLabs, 2017), 4) haemagglutinin (HA) antigen or virus-like particles grown in insect cells, and 5) defective-replicating alphavirus (dα-H5; equine encephalitis virus) with a H5 AI virus gene insert (Swayne and Sims, 2021).

The most commonly employed vaccines are derived from inactivated whole viruses, although the introduction of new vaccine antigens can be slow and lag behind the development of antigenic changes in field viruses. Inactivated vaccines can induce a good humoral response but this can be transient and there is poor stimulation of cellular immunity (Nurzijah *et al.*, 2022; Swayne and Sims, 2021), possibly related to the need to administer them parenterally (Calzas and Chevalier, 2019).

Vector vaccines may also be valuable in mass spray applications for emergency vaccination because vector viruses such as Newcastle disease virus are naturally effective at penetrating the mucosal surface. They stimulate humoral and cellular immunity, but a previous infection with the vector and existing immunity can interfere with vaccine immune response and multiple doses may be needed. Subunit vaccines have challenges with low immunogenicity and still need direct inoculation (Nurzijah *et al.*, 2022; Swayne and Sims, 2021).

Experimental vaccine technology includes live virus vaccines, haemagglutinin produced in plants or cell cultures and naked DNA vaccines. However, new vaccines will have to have a competitive cost (not achievable by DNA vaccines), offer better protection and aid mass vaccination, to have a chance at commercialisation. Live AIV vaccines must not revert to virulence and should therefore be prevented from replicating, but a single dose may be sufficient and cellular immunity is stimulated. However, DNA vaccines may not evoke an effective humoral response (Nurzijah *et al.*, 2022; Swayne and Sims, 2021).

8.3.1. Virus-like particle (VLP) vaccines

In vitro-produced recombinant antigen vaccines, synthesised in plant leaf tissue, insect or bacterium cells, offer alternatives to conventional inactivated avian influenza vaccines (Balke and Zeltins, 2020; Swayne *et al.*, 2014). Though vaccine production is relatively complicated (Nurzijah *et al.*, 2022), rapid synthesis of many doses is possible once a field virus has been genetically sequenced and no live virus is required, which increases safety (Abolnik *et al.*, 2022b). The imitation haemagglutinin protein on a virus-like particle (VLP) is highly ordered in a dense array similar to a live virus, which interacts with antigen-presenting cells and stimulates a strong cellular and humoral response (D'Aoust *et al.*, 2010; Smith *et al.*, 2019). The absence of internal virus proteins, like the nucleoprotein (NP), allows for DIVA using standard ELISA tests. VLP-vaccines against human papillomavirus and hepatitis B have been registered, but none are registered for influenza viruses or for animal use (Chu and Quan, 2023).

Plant production of VLPs has advantages including lower cost, compared to insect cell cultures and microbial fermentation, and it is faster, more scalable and safer than insect cell systems. Low-alkaloid tobacco plants are commonly manipulated to generate pharmaceuticals because it is easy to insert

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foreign genes, which are then expressed in a stable manner, and they grow fast and prolifically, producing large quantities of recombinant proteins. There is also the advantage that they are not produced for food and so are unlikely to enter the food chain (Lee *et al.*, 2023).

Smith *et al.* (2019) tested a tobacco leaf-derived VLP H6 vaccine, also containing the matrix 2 (M2) protein, in chickens for the first time. They found that a single 768 HAU dose in 6-week-old chickens produced comparable antibody titres to two doses of the inactivated vaccine and significantly reduced virus shedding after infection. The M2 protein was included to increase VLP production. The group further explored the effect of dose and adjuvant, using an updated H6 VLP (Abolnik *et al.*, 2022b). VLPs without adjuvant produced haemagglutination inhibition assay (HI) titres >32 only after a booster. The 250 HAU dose produced the highest titres by 3 weeks after the initial vaccination but there were no significant differences in the months afterwards between 125, 250 and 500 HAU and between 250 HAU with 25% and 50% adjuvant.

Abolnik *et al.* (2022a) vaccinated layer hens with a tobacco leaf-produced clade 2.3.4.4 H5 VLP from a codon-optimized DNA sequence based on SA field strain A/Speckled pigeon/08-004B/2017. The M2 protein was again included. They compared four adjuvants, with three vaccines injected intramuscularly and one applied as an eye drop. The three injected vaccines produced mean $\log_2$ titres of between 7.3 and 8, three weeks after a second dose, whereas the intraocular vaccine only produced a titre of 2. The hens that received the vaccine with the oil-in-water adjuvant and polymer immunostimulant, that performed best, were challenged with homologous virus. They were completely protected from clinical signs and had significantly reduced virus shedding compared to the control group. A kilogram of leaves would be sufficient for more than 85 000 doses (Abolnik *et al.*, 2022a). The authors reported no local or systemic vaccine reactions. Additionally, Rutkowska *et al (*2019), who tested a VLP African horse sickness vaccine, produced in tobacco plants at the same facility, found it safe with no lesions at the injection site and no side effects.

Studies in humans have provided further evidence of the safety and immunogenicity of influenza A tobacco-produced VLP vaccines. Ward *et al.* (2014) tested H5 and H1 tobacco-produced VLP vaccines and reported that none of their 280 vaccinated subjects developed allergic or hypersensitivity symptoms, even though 40 had pre-existing plant allergies. Adverse effects reported from the other studies were mild to moderate. They commonly included pain and tenderness at the injection site (Cummings *et al.*, 2014; Landry *et al.*, 2010; Pillet *et al.*, 2019) and Pillet *et al.* (2019) found that the incidence increased with dose. Pillet *et al.* (2019) also reported systemic reactions including mild and transient headache, malaise, and fatigue. Cummings *et al.* reported 5% vaccine-related headaches but a similar proportion of subjects experienced adverse effects in treatment, control, and approved vaccine groups.

Cummings *et al.* (2014) compared three doses of a tobacco-produced H1N1 haemagglutinin vaccine, unadjuvanted or adjuvanted with aluminium hydroxide. The group that received the highest (90µg) dose of unadjuvanted vaccine had antibody titres comparable to the licensed vaccine by day 56, but a booster dose was required, unlike the licensed vaccine.

Landry *et al.* (2010) showed an H5N1 H5 alum-adjuvanted tobacco-produced VLP prevented pathology and reduced shedding in ferrets after challenge and they compared three doses in humans. The highest dose (20 µg) induced seroprotective levels in 50% of subjects, 3 weeks after the second vaccination.

Pillet *et al.* (2019) tested a quadrivalent (HA proteins from two influenza A, H1N1, H3N2, and two influenza B viruses) tobacco-produced VLP vaccine, at different doses, and investigated an alum adjuvant in subjects older than fifty, which seemed to have little effect. They reported >80% seroprotection from all doses in younger adults (<50 years), when using HI tests based on the different viruses, and more than 70% in most groups of older adults, for most viruses.

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8.3.2. Determinants of vaccine immunogenicity

The antigenic content of an avian influenza vaccine and the adjuvant (Crawford *et al.*, 1999), as well as antigenic match to the challenge virus, contribute to immunogenicity. The antigenic mass must also be matched to the body mass of the recipient (Koch *et al.*, 2009). Antigenic content can be measured directly or indirectly, using various methods. Indirect quantification methods include the level of haemagglutination caused by the HA antigen (haemagglutination units, HAU). HAU is only an indication of antigenic content, however, and may not always correlate with the mass of protein present, i.e. with results of a direct method.

Studies of the optimal antigenic content of AIV vaccines, reporting antigenic content as HAU, have suggested a range of ideal doses, from 128 to 640 HAU. It appears that, in cases where the vaccine and challenge virus strains were the same, 250 HAU is the optimal dose required in chickens (Crawford *et al.*, 1999; Kilany *et al.*, 2016; Maas *et al.*, 2009; Sasaki *et al.*, 2009; Spackman *et al.*, 2014).

Adjuvants can be added to or built into vaccines to improve their performance in a variety of ways, including increasing the magnitude and duration of immune response (Calzas and Chevalier, 2019). Adjuvants comprise a wide range of chemicals and compounds but a common function is to stimulate the immune system appropriately, to ensure an adequate response to the vaccine's active ingredient (Swayne and Kapczynski, 2008). Virus-like particle vaccines can display additional proteins that assist with stimulating the immune cells and ensure a robust response (Chu and Quan, 2023). Mineral oil adjuvants increase antibody production, partly by delaying the release of vaccine and prolonging the immune response (Swayne and Kapczynski, 2017).

The characteristics or physiological status of the recipient also affect the immune response to a vaccine. Examples include sex, age, genetic factors, physiological stress, nutrition and immunosuppression caused by concurrent infection (Swayne and Sims, 2021; Van Loveren *et al.*, 2001). Environmental factors known to affect immune response in poultry include toxin exposure, water deprivation, excessive heat and cold and overcrowding, and could also affect response to vaccination (Cheville, 1979). Maternally-derived antibodies in young animals can also suppress antibody response to vaccines (Staszewski *et al.*, 2007; Zimmermann and Curtis, 2019).

9. African Penguin biology relevant to disease risk and transmission

9.1. Distribution and population size

African Penguins occur only in southern Africa, nesting on islands and in two mainland colonies along the coastline from central Namibia to Algoa Bay, in the Eastern Cape Province of South Africa. Three regional populations exist: in Namibia, the Western Cape province of South Africa and Algoa Bay (Ryan, 2017). The South African population was estimated at 16 000 breeding pairs in 2018 (Makhado *et al.*, 2018) with the only other population being approximately 5000 pairs in southern Namibia. These numbers of breeding pairs translate to a total number of approximately 67 200 individuals, calculated by multiplying the estimated breeding pairs in 2017 by 3.2 (Crawford and Boonstra, 1994). African Penguin numbers have decreased by more than 70% over the last 40 years due to factors such as declining fish stocks, especially on the west coast, and unsuitable breeding areas (Birdlife International, 2018).

9.2. Breeding season

Breeding occurs year-round, though there are differently-timed peaks at different colonies. Usually, only one clutch of two eggs is laid by a pair, but some attempt and may raise a second clutch. Not all pairs will breed every year and food availability seems to play a role. Eggs are laid in burrows, rock crevices or under vegetation and both parents take turns incubating the eggs for approximately 40

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days. The chicks fledge after approximately 80 days, so breeding adults will spend at least four months on incubation and chick-raising (Crawford and Whittington, 2005; Ryan, 2017).
Chicks are brooded under a parent until approximately 10 days (classified by plumage as "P1") and develop secondary down by about 20 days (P2), so they can be left alone by approximately a month, when multiple chicks may form crèches. They start to lose down and develop feathers at about 40 days old (P3) and may have their grey and white juvenile plumage by about 60 days (classified as a "blue"). A P4 chick has lost at least half its down. On average, a pair will fledge a maximum of 0.67 chicks per year. Before the age of a month, chicks are more likely to die of exposure or accidents such as burrow collapse or flooding. Older chicks are more likely to die from starvation (Crawford and Whittington, 2005).

9.3. Movements

Fledglings ("blues") leave the colony and remain at sea for one to two years before they return to a colony to moult into adult plumage (Kemper, 2015). Juveniles from the Western Cape colonies usually move north towards Namibia after fledging and some go as far as a feeding site around Swakopmund, halfway up Namibia's coast, others to an area north of St Helena Bay and others (from the Eastern Cape) to an area around Cape Agulhas (Sherley *et al.*, 2017). Flipper band recoveries and resightings show that some birds may visit colonies in other regions (PA Whittington *et al.*, 2005). After their first moult, juveniles may visit various colonies before choosing one for breeding. Adults from the Western Cape will not travel further than 30 km away from the colony while feeding chicks but can also disperse north (as far as the Orange River mouth) or east when not breeding. Approximately half of the birds from Dassen Island may even undergo their annual moult at Stony Point. However, adults from the Eastern Cape seem to remain closer to the colony during the non-breeding season and most seem to moult on Bird Island, though they may hunt as far east as East London (Carpenter-Kling *et al.*, 2022; Roberts, 2016). Non-breeding birds may move between colonies in Namibia (Kemper, 2015). While out at sea, African Penguins may feed with Sooty Shearwaters, Cape Gannets (*Morus capensis*), Cape Cormorants, Kelp Gulls, terns and marine mammals (Crawford and Whittington, 2005).

9.4. Moult

The annual catastrophic moult involves the penguin losing and replacing all its feathers over approximately two weeks. As the bird is not waterproof and cannot swim to feed during the process, there is a pre-moult fattening period to prepare. Birds gain approximately an extra third of their weight and lose it again, and often more, over the moult. Historically, most of the birds moulted in the inter-breeding period. This is generally between October and December (Ryan, 2017) but some colonies moult later in summer and in Namibia they moult in autumn (Crawford and Whittington, 2005). However chick-raising periods seem to be overlapping increasingly with moulting at some colonies, and chicks starve if their parents cannot swim to catch them food (Sherley *et al.*, 2014b).

9.5. Population structure

African Penguins first breed when they are approximately four to six years old, but usually at five years (Crawford and Whittington, 2005; Philip Whittington *et al.*, 2005) and are known to live at least 27 years (Whittington *et al.*, 2000). Lifespan is commonly stated as 10 to 11 years, possibly based on an annual adult survival rate of approximately 70%, but estimated survival rates have varied widely over time (they have generally decreased) and by location (Crawford *et al.*, 2011; Kemper *et al.*, 2007; Sherley *et al.*, 2014a).

9.6. Colonies and breeding sites

African Penguin colonies vary widely in location, breeding season, climate, vegetation, nest density and neighbouring species. Many of the islands were once covered in deep layers of seabird guano,

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into which African Penguins burrowed to nest, but the guano was harvested and the penguins now take advantage of whatever shelter is available.

In the Western Cape province of South Africa, African Penguins nest at five main coastal sites, within the winter rainfall area of South Africa. The two mainland colonies are in the towns of Simon's Town, accommodating the Boulders, part of Table Mountain National Park, and in Betty's Bay, including Stony Point provincial nature reserve. Both colonies were established in the 1980s. Robben and Dassen Islands are on the west coast, while Dyer Island holds the largest eastern-most colony. Peak breeding occurs in winter at most colonies, but in summer on Dassen Island and more in autumn on Bird Island (McInnes, 2016).

Espinaze *et al.* (2019) estimated nest density at the main Western Cape colonies by counting nests in random 15m x 15m quadrants each year for the two years of their study (2016 and 2017). Both inactive and active (containing eggs, chicks or adults) were counted, but differentiated. The maximum total nest (inactive and active nests) density was 0.28 nests/m^2 at Stony Point and the maximum active nest density was at Simon's Town (0.14 nests/ m^2). A large number of artificial nest boxes have been installed at both sites, but penguins also nest under bushes and rocks and in burrows, where there is suitable soil. There is also a relatively dense group of surface nests on Foxy Beach at Boulders. Other seabird species such as gulls, terns and cormorants visit and roost at both sites but Stony Point also accommodates breeding Bank (*Phalacrocorax neglectus*), Cape (*P. capensis*), Crowned (*P. coronatus*) and White-breasted Cormorants (*P. lucidus*) (capenature.co.za) and Egyptian Geese, which, along with Sacred Ibis, have increased along the Western Cape coast in recent decades (Ryan, 2013).

Dyer Island has an intermediate nest density (0.08 total and 0.06 active nests/ m^2), with many nests in a variety of artificial boxes installed over the years. In addition to the penguin colony, a large number of Cape Cormorants, Hartlaub's gulls and Swift Terns, as well as smaller numbers of other cormorant and tern species breed and roost there (birdlife.org.za). Historically, Dyer Island was the site of multiple *Pasteurella multocida* (avian cholera) outbreaks, which occurred every year between 2002 and 2005 (Waller and Underhill, 2007).

Espinaze *et al.* calculated the minimum total nest density for the Western Cape on Dassen Island (0.02 nests/ m^2) and the minimum active nest density on Robben Island (0.007 nests/ m^2). These are the larger islands and both are on the west coast: Robben Island is in Table Bay, north of Cape Town, and Dassen another 50 km to the northwest. Dassen Island is generally flat and sandy, with dispersed penguin nests in burrows or under *Tetragonia fruticosa* bushes inland, and under rocks along the coastline. Dassen also accommodates one of two South African Great White Pelican breeding sites, breeding cormorants, Kelp and Hartlaub's Gulls and Swift Terns. Migratory waders such as Ruddy Turnstones, are present in summer (birdlife.org.za). Robben Island has been occupied by humans since the 1500s and penguins nest mainly under alien vegetation. The island also hosts a large Cape Cormorant colony and important populations of Bank Cormorant, Hartlaub's Gull and Swift Tern and a large Kelp Gull colony.

Subjectively, nest density on Bird Island, in the Eastern Cape's Algoa Bay and in the summer rainfall area, approximates that of Stony Point and Boulders (in occupied areas). St Croix Island historically had the larger colony in Algoa Bay, when Algoa Bay held half the global African Penguins population. However, the St Croix colony has drastically reduced in recent years, possibly due to increased shipping traffic and noise (Pichegru *et al.*, 2022). Bird Island is flat and sandy, and half is occupied by the largest Cape Gannet breeding colony in South Africa, nearly 100 000 pairs (Sherley *et al.*, 2019). Many penguin nests are in artificial nest boxes but there is also an area of burrows, some nests in disused buildings and others under low vegetation.

Halifax and Possession Islands are on the south coast of Namibia, near Lüderitz, and adjacent to the Namib Desert. Though rain falls very seldom, there is frequent fog (100-125 days a year) that creates

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damp and cool conditions (annual mean temperature less than 16°C; Portik *et al.*, 2010). It was estimated that Halifax and Possession Islands accommodated 27% and 17% of the Namibian population respectively in 2017, when the total population was recorded as approximately 5500 breeding pairs (Ministry of Fisheries and Marine Resources, unpublished data). The majority of the Namibian population has historically bred further north on Ichaboe and Mercury Islands. On Halifax, the penguins breed in five dense colonies, mostly in open nests formed by slight hollows, approximately 0.5m apart. The nests are on average more widely dispersed on the much larger Possession Island (more similar to Dassen Island) and more are found under sparse, low bushes and in earth burrows, but there are also some dense open colonies similar to Halifax. The main breeding season is over spring/ summer but there is a secondary winter peak (Kemper, 2015).

9.7. African Penguin body condition indices

In order to avoid the subjectivity of body condition scoring, Lubbe *et al.* (2014) and Campbell (2016b) performed quantile regression to formulate body condition indices for African Penguin chicks and adults respectively, based on head length (75mm or more) and bill length, respectively. A calculated index between 0 and 1 indicates that the bird's mass is between the 0.95 and 0.05 quantiles, an index greater than one means the bird has a mass over the 0.95 quantile and a negative index indicates a mass below the 0.05 quantile.

Lubbe *et al.* derived their index from data obtained from regular measuring of chicks until fledging.

Campbell used body mass and bill length from 1376 wild adult African Penguins to perform quantile regression and validated the resulting body condition index (BCI) against a body condition assigned by a vet on a post-mortem examination of 133 dead penguins. The vet assigned body condition as “emaciated”, “thin”, “moderate”, “good” or “fat”, depending on carcass fat and prominence of the keel bone. Birds assessed as “fat” had a mean BCI of 1.052 and the mean BCI for “thin” birds was 0.138. Campbell also discusses the potential for prey load to affect body mass and therefore the accuracy of the BCI.

Cooper (1978) showed that approximately half the weight lost between pre- and post-moult is water. It is therefore possible that body mass in moulting penguins is not necessarily a reliable indicator of fat and protein content (unless they gain water at the same rate as fat and protein) and that the abovementioned BCI for adults may not be applicable.

9.8. Sex determination from beak shape in adult African Penguins

Male African Penguins have larger bills (Duffy, 1987) but also relatively deeper bills than females. Other than a slightly larger body size, this is one of the only ways to determine sex without genetic testing. Campbell *et al.* (2016) derived the “Farah discriminant function”. This, when applied with two cutpoints, correctly identified the sex in 97% of South African Penguins also sexed by DNA analysis, though some birds with intermediate bill measurements were classified as “sex unknown”. Although the function was not validated in Namibian penguins, Duffy (1987) compared male and female beak dimensions between colonies in Namibia and South Africa. The only significant difference in bill size between colonies was in male beak length between the two most distant islands assessed (Mercury and Dyer, with larger beak length at Mercury). The Campbell function was therefore expected to be valid in Namibian penguins.

10.Citizen science and coastal monitoring

Citizen science makes use of members of the public who volunteer to assist with gathering scientific information. It offers an opportunity to collect data on a much larger scale than is possible by a single individual or even a team of researchers, and at a much lower cost. It is also a valuable tool to educate

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the public, involve them in conservation and encourage scientific thinking. The term "citizen science" was introduced in 1995, though the concept had been around much longer (Bonney *et al.*, 2009). The use of citizen science has continued to expand and evolve and the development of, especially, mobile phone technologies has made it possible to include more volunteers and gather more accurate data (Catlin-Groves, 2012). There has been valuable research into the reasons people participate in citizen science projects (Land-Zandstra *et al.*, 2016; Wright *et al.*, 2015) and determinants of success and quality of citizen science research (Bonney *et al.*, 2009; Frigerio *et al.*, 2018; Tweddle *et al.*, 2012) to help address concerns about data collected by non-scientists. For example, lack of visualization of data has been shown to reduce participation (Bonney *et al.*, 2009; Catlin-Groves, 2012).

Lawson *et al.* (2015) reviewed the use of citizen science for wildlife disease surveillance. They discuss a targeted syndromic approach, where volunteers observe for particular clinical signs to target specific pathogens, and opportunistic reporting, which is more *ad hoc*. However, more valuable data can be obtained with a systematic approach, which could include organised transect surveys. The authors found that citizen science disease surveillance programs benefit from building on existing citizen science networks.

Citizen science has long been employed to study birds (Wright *et al.*, 2015) and projects exist that can be built upon or at least consulted, to assist with beached bird surveys. The National Audubon Society in the USA had their first annual Christmas bird count in 1900 and, with the Cornell Lab of Ornithology (CLO), runs eBird (eBird.org). eBird is a citizen science project, linked to an app that allows collection of data, location and method of birdwatching and then a checklist of bird species seen. It provides a tool for birdwatchers, but also provides data for the study and monitoring of bird distribution, abundance, habitat use and trends. CLO also runs other shorter-term citizen science projects such as the House Finch Disease Survey, which surveyed conjunctivitis caused by *Mycoplasma gallisepticum* (Bonney *et al.*, 2009).

Large ornithological citizen science projects in southern Africa include the Southern African Bird Atlas Project 1 (SABAP1) that ran from 1987 to 1991 and provided distribution data for bird field guides, and SABAP2, that began in 2007. As of July 2019, SABAP2 had over 17 million records contributed by hundreds of volunteers. SABAP2 is based at the University of Cape Town (UCT) and is funded by the FitzPatrick Institute of African Ornithology and the South African National Biodiversity Institute (SANBI) and is supported by BirdLife South Africa and BirdLasser (sabap2.adu.org.za/). BirdLasser (originating from "bird atlas") is a mobile app for recording bird sightings, developed and launched in 2014 by South Africans (Walker, 2014) to assist SABAP2.

Both SABAP1 and SABAP2 were originally launched and administered by the Animal Demography Unit (ADU) at UCT, which was also responsible for The South African Bird Ringing Unit (SAFRING) and the Co-ordinated Waterbird Counts (CWAC), running since 1992 and monitoring 400 wetlands in South Africa (cwac.adu.org.za/).

SANBI also supports other citizen science projects and uses iNaturalist as an online platform to support projects and collect data. iNaturalist allows observations of plants, animals, fungi and any other living organism to be added to and contribute to any number of projects. One is a coastal strandings project (SANBI Biodiversity Advisor, 2019).

Stranded marine mammals (e.g. whales, seals, and dolphins), reptiles (e.g. turtles) and seabirds are reported to various governmental groups or non-governmental organisations who collate reports and, where possible, rehabilitate animals that are still alive. For example, in 2016, 76 incidents were reported by the Kwa-Zulu Natal Strandings Network, which included ten whales, eight dolphins, five seals and nine turtles, the remainder being seabirds (Olbers, 2017).

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Beached bird surveys have long been used to monitor mortality in seabirds and provide a stimulus for further investigation, monitoring or intervention. Such surveys have proved to be invaluable, however, the value of data collected depends greatly on the survey objective and design, volunteer willingness and coordination, and measurement parameters (Camphuysen and Heubeck, 2001).

Oceans are under considerable anthropogenic and climate-related threats and monitoring seabirds has also become an important tool for evaluating ocean health (Parsons *et al.*, 2008). Seabird surveys have been successfully used elsewhere for this purpose. For example, three beached bird citizen science programs: Beach Coastal Ocean Mammal/Bird Education and Research Surveys (Beach-COMBERS), Beach Watch, and the Coastal Observation and Seabird Survey Team (COASST) documented a mass die-off of Common Murres. This was observed in the north-west Pacific along the coast of North America from California to Alaska from summer 2015 to spring 2016 and has been linked to a marine heatwave from 2014 to 2016. A mass of warm water off the coast of California to Alaska exceeded average temperatures by 2-3 standard deviations. This may have resulted in a decrease in the murres' prey fish and starvation of the birds (Piatt *et al.*, 2020).

11.Contingency planning

Contingency plans for disease outbreaks in domestic animal species are common and guidelines are provided by most international organisations (FAO, 2011; OIE, 2018; TemaNord, 2014; Westergaard, 2008; WHO, 2018). The basic structure and approach are similar in wild animal outbreaks, in terms of providing accessible information on organisation structures, relevant legislation, responsible parties to be consulted, communication guidelines, and details of risk assessment, preparation and response. One example is provided by Wildlife Health Australia in their "Guidelines for management of an emergency wildlife disease response (working draft November 2018)".

The guidelines on responding to an outbreak should include more technical information, for example about identifying the cause of the outbreak, in terms of sample collection and laboratory testing, and then about options to control the outbreak. Parsons and Vanstreels (2016) provide a good overview of technical aspects to be considered when managing a disease outbreak in a seabird colony, which will be valuable when formulating a template for contingency plans for specific sites.

The United States Geological Survey has published a Field Manual of Wildlife Diseases that is another valuable reference. The 1999 (second) edition (Friend and Franson, 1999) was subtitled "General Field Procedures and Diseases of Birds". The bulk of the document covers important bird diseases and the "Procedures" included sample collection and transport, recording history data and "Disease Control Operations". The third edition has been published as a web page, "Field Manual of Wildlife Diseases: Techniques and Methods 15" (Franson *et al.*, 2015) and sections on mortality investigation, specimen collection etc. have been updated.

The Ramsar Wetland Disease Manual (Cromie *et al.*, 2012) covers some generally relevant aspects such as disease investigation, and disease management including principles of carcass collection and disposal and vaccination. There is a section specifically on avian influenza but although some mass mortality had occurred in wild birds, the document pre-dates any recognised need for management in wild birds. The Ramsar handbook "Avian influenza and wetlands" (Ramsar Convention Secretariat, 2010) most usefully provides guidance on surveillance for and early detection of HPAI and a list of considerations for contingency planning, as well as a list of other sources of information (mostly for poultry) but practical details are few.

A pre-conference workshop on mass mortality events in penguins was held at the 10th International Penguin Conference in August 2019. A conclusion of the workshop was that there is a need for

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guidelines for investigating mortality events in seabird colonies and for sharing information and managing outbreaks.

Since HPAI outbreaks intensified in seabirds worldwide in 2022, many organisations have developed guidance documents to assist with planning for HPAI in wild bird colonies, and countries have developed protocols and contingency plans.

Examples of guidance documents:

- Scientific Task Force on Avian Influenza and Wild Birds statement on H5N1 high pathogenicity avian influenza in wild birds - unprecedented conservation impacts and urgent needs (CMS FAO Co-convened Scientific Task Force on Avian Influenza and Wild Birds, 2023)
- World Organization for Animal Health's Working Group on Wildlife guidelines for "Risk management for people working with wild birds" (Uhart *et al.*, 2022)
- The Agreement on the Conservation of Albatrosses and Petrels (ACAP) Intersessional Group on High Pathogenicity Avian Influenza H5N1 "Guidelines for working with albatrosses and petrels during the high pathogenicity avian influenza (HPAI) H5N1 panzootic (Serafini *et al.*, 2023)

Examples of response/ contingency plans:

- Phillip Island Nature Parks Avian Influenza Wildlife Response Plan (Renwick, 2023)
- Management guidelines - Mitigation and data collection strategies for avian influenza in bird colonies in the Wadden Sea (Bregnballe et al., 2023).
- Mitigation strategy for avian influenza in wild birds in England and Wales (DEFRA, 2023)
- Scottish Wild Bird HPAI Response Plan (Scottish Government, 2023)
- "United Kingdom procedures for preventing the introduction or spread of Highly Pathogenic Avian Influenza in Antarctica" (United Kingdom, 2023)
- Highly Pathogenic Avian Influenza (HPAI) and Wild Animals in Australia (Wildlife Health Australia, 2023)
- Government of South Georgia & the South Sandwich Islands' "Biosecurity Handbook" updated to include a section on "Special measures for Highly Pathogenic Avian Influenza" (Government of South Georgia & the South Sandwich Islands, 2023).

12.Conclusion

Despite reasonably plentiful information on different aspects of HPAI viruses' biology, epidemiology and capabilities, the interactions with different hosts and environmental conditions make the disease highly unpredictable still. This is especially the case in southern Africa, where the presumed natural hosts of the virus, the ducks and geese, do not move predictably or move according to environmental cues that are not fully understood. However, given growing evidence of virus introductions via West Africa from Europe, virus detections in West Africa can be monitored to plan outbreak response in southern Africa. Unfortunately, avian influenza surveillance in Africa may not be reliable and there is a need to strengthen avian influenza detection capabilities at key sites where wild ducks and geese congregate. Early warning of HPAI outbreaks will ideally allow stakeholders to prepare their staff and systems according to pre-determined preparedness plans, to monitor for outbreaks and respond efficiently. Much of this response may be simply monitoring and responding to questions from the public, unless an effective management tool emerges. The most likely candidate is a vaccine, but it needs to be in a formulation that can be feasibly applied to wild birds, and it needs to be applied in a targeted manner to be most effective. It may be possible to develop effective vaccines that can be safely applied to and protect endangered species, but if they do not play a key role in virus spread, there may be another species that can be vaccinated to greater effect. This knowledge only comes from understanding of different species roles in the disease's epidemiology.

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Chapter 3

Descriptive epidemiology and response to the clade 2.3.4.4 high pathogenicity avian influenza epidemics in South African coastal seabirds, 2018 and 2021/22

In part adapted from Roberts, L.C., Abolnik, C., Waller, L.J., Shaw, K., Ludynia, K., Roberts, D.G., Kock, A.A., Makhado, A.B., Snyman, A., Abernethy, D., 2023. *Descriptive Epidemiology of and Response to the High Pathogenicity Avian Influenza (H5N8) Epidemic in South African Coastal Seabirds, 2018*. Transbound. Emerg. Dis. 2023, 1–13. https://doi.org/10.1155/2023/2708458 [1]

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1. Introduction

From approximately 2016, avian influenza Goose/Guangdong (Gs/GD)-lineage clade 2.3.4.4b H5N8 viruses caused the fourth and, until then, most severe global high pathogenicity avian influenza (HPAI) wave. Until then, it was the most severe HPAI panzootic in terms of the number of countries, farms and zoos and wild birds affected (Sims *et al.*, 2017). The first detection was in wild water birds in May 2016 in Russia and China, and the virus subsequently spread across Asia and Europe and into Africa over the following year. Europe experienced several mass mortality events in wild waterfowl (European Union Reference Laboratory for Avian Influenza (EURL), 2018, 2017; Kleyheeg *et al.*, 2020; Meier *et al.*, 2017; Muzyka *et al.*, 2019; Napp *et al.*, 2018). In Africa the deaths of 60% of 2000 White-winged Black Terns (Chlidonias leucopterus) in early January 2017 on Lake Victoria in Uganda were attributed to H5N8 HPAIV (FAO, 2017; Khomenko *et al.*, 2018; Ndumu *et al.*, 2018). In June 2017, HPAI (H5N8) reached South Africa, causing widespread outbreaks in commercial poultry, backyard/ hobby birds and terrestrial wild birds until October 2017 (Khomenko *et al.*, 2018). At least nine seabirds, including six African Penguins (*Spheniscus demersus*), were tested as suspected HPAI cases between September and November but no virus was detected (Western Cape Veterinary Services unpublished data). At the end of December, HPAI (H5N8) was detected in a Swift Tern (Thalasseus bergii), marking the first detection in a southern African seabird, and mass mortalities in Swift Terns and detections in other seabird species followed in the first half of 2018.

Five distinct genetic variants of the HPAI (H5N8) virus were detected in the northern half of South Africa in 2017 but only one of these reached the southern provinces, detected two months after the first cases in the north (Abolnik *et al.*, 2019). Phylogenetic and time-to-the-most-recent-common- ancestor analyses on coastal bird viral genomes revealed that the HPAI virus affecting coastal birds most likely emerged in October 2017, from a virus circulating in the southern provinces (Peyrot *et al.*, 2022). These viruses were, in turn, the ancestors of viruses causing mass mortality in African Penguins in Namibia in early 2019 (Peyrot *et al.*, 2022; Umberto *et al.*, 2020). West Africa, with its extensive permanent wetlands, acts as the central hotspot for virus introduction and dissemination in the continent, and genetic data indicate that the 2017 H5N8 HPAI outbreaks in South Africa were most likely introduced from West Africa with intra-African nomadic ducks (Abolnik, 2019; Fusaro *et al.*, 2019). Locally, ubiquitous bridge species such as Egyptian Geese (*Alopochen aegypticus)* and Sacred Ibis (*Threskiornis aethiopicus*) could have introduced the virus to coastal species.

Clade 2.3.4.4b H5N1 viruses were introduced to West Africa in late 2020 (Lo *et al.*, 2022; Meseko *et al.*, 2023), though significant numbers of H5N1 outbreaks only began outside Africa from October 2021 onwards (Abolnik *et al.*, 2023). The first detection of HPAI (H5N1) was made in South Africa in April 2021 and continued into 2023. This time, the initial cases in seabirds were detected at a similar time to those in domestic poultry, as were outbreaks in different parts of the country, but mass mortalities in seabirds only occurred from September onwards. The viruses in southern Africa were linked to those detected in West Africa in late 2020/ early 2021 and were genetically distinct from those detected in Europe and Asia (Abolnik *et al.*, 2023).

The devastating outbreaks in seabirds in South Africa in 2018 and 2021 and in the northern hemisphere and South America in 2022 and 2023 (Alexandrou *et al.*, 2022; Grémillet *et al.*, 2020; Knief *et al.*, 2023; Leguia *et al.*, 2023; Lublin *et al.*, 2023) have highlighted the need to share and consolidate knowledge about preparedness and response measures required to manage such events. This chapter describes the distribution of clade 2.3.4.4b H5N8 and H5N1 HPAI in coastal seabirds in South Africa in 2018 and in 2021 and 2022. Morbidity and mortality were reported by fieldworkers who were clearing carcasses, members of the public, rehabilitation centres and researchers, among others. Proportions of samples in which virus was detected are reported per species, to indicate the probability that mortalities were due to HPAI virus. This chapter also describes the response to the outbreaks, including communication and coordination, monitoring of the outbreak, and the response in the wild and in rehabilitation centres. It discusses the lessons learnt, among which is the need for preparedness plans.

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2. Material and Methods

2.1. Case Definitions

A suspected HPAI case was defined as an individual wild bird with clinical signs indicative of avian influenza or found as a carcass with no other apparent cause of death between 1 December 2017 and 31 May 2018 and from 1 May 2021 to 31 December 2022. Indicative clinical signs included abnormal behaviour, such as depression and lack of normal response to stimuli, and more obvious neurological signs such as ataxia, circling, torticollis and tremors. Birds reported to L Roberts as simply "sick" or "infected" were included but birds with injuries were excluded. Initial suspected cases from new species and sites were prioritised for sampling and testing, to save limited government resources. However, anyone could submit samples and pay for testing, so sampling was not always restricted in this way. Research funding had been sourced by 2021, and was available for testing seabird carcasses (approximately 240) and live African Penguins admitted to the SANCCOB rehabilitation centre in Table View (n=382).

A confirmed or "positive" case was defined as an individual bird from which an organ sample, brain, cloacal or tracheal/oropharyngeal swab sample or any pooled samples tested positive on influenza A virus (IAV) real-time reverse transcription polymerase chain reaction (rRT-PCR) and H5-, N8- or N1-specific rRT-PCR, or was confirmed as H5 HPAIV via Next Generation Sequencing (Abolnik *et al.*, 2023; Peyrot *et al.*, 2022). Suspected cases that then tested IAAIV negative were no longer counted as suspected, but IAV-positive samples that were negative on further tests were retained as suspect cases, due to suspected poorer sensitivity of the H5 and HP H5 PCR test, and the assumed low probability that a sick or dead bird infected with IAV during the outbreak would be infected with another subtype. The suspicion was supported by work published by Abolnik *et al.* (2024).

2.2. Sample collection

Samples were collected by veterinary or research staff at seabird rehabilitation centres, state veterinarians at the Western Cape Provincial Veterinary Laboratory (WCPVL) and occasionally by private vets and field staff. Samples and carcasses were submitted to the WCPVL, or in 12% of positive 2018 cases and 62% of positive 2021/22 cases, to a private laboratory Deltamune (later AssureCloud). Brain samples were obtained by making a small incision in the skin and skull on the top of the head and inserting and agitating a cotton swab with a plastic handle to collect brain tissue.

2.3. Laboratory testing

At the Western Cape Provincial Veterinary Laboratory (WCPVL), the influenza A rRT-PCR was performed using the VetMAX™Gold AIV detection kit (Thermo Fisher: Life Technologies, Cat. no. 4485261) according to the manufacturer's protocol. The kit targets the matrix protein 1, matrix protein 2 and nucleoprotein genes of influenza A viruses. Cycle threshold (Ct) values < 36 were considered positive. The VetMax-Plus One-Step RT-PCR Kit (Cat. No. 4415328) was used for the H5 and N8 tests.

At AssureCloud private laboratories, samples were screened using the rRT-PCR RealTime ready RNA Virus Master kit (Roche Life Science, Cat. No. 05992877001), targeting the IAV matrix gene with primers and probes described by Spackman *et al.* (2002), supplied by TIB Molbiol Synthesis Laboratory, Berlin, Germany (Ref: 022111063). Ct values <35 were considered positive.
AIV H5 subtype detection was carried out using the primers and probes described by Slomka *et al.* (European Union Reference Laboratory for Avian Influenza and Newcastle Disease, 2021.; Slomka *et al.*, 2007) and Ct ≤ 35 was considered positive. AIV N8 detection was performed using primers and probes described by Hoffman *et al.* (European Union Reference Laboratory for Avian Influenza and Newcastle Disease, 2021; Hoffmann *et al.*, 2016) considering Ct ≤ 36 as positive.

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As required by the South African national Directorate: Animal Health, all AIV-positive samples were forwarded to the Agricultural Research Council's Onderstepoort Veterinary Research Institute and confirmatory and sub-typing tests were performed. RNA from HP H5 AIV was detected using real-time RT-PCR (primers and probes: Naguib *et al.* (2017)), and RNA from N1 AIV was detected using SYBR Green-based real-time RT-PCR (primers: Payungporn *et al.* (2006)).

2.4. Outbreak monitoring and reporting

Identification of suspected cases relied on reports submitted by the public when they happened to observe suspicious birds, by veterinarians at rehabilitation centres or by staff of the managing conservation authorities. These staff reported observations made either during scheduled patrols or during other daily activities. Daily monitoring took place at one of the mainland penguin colonies and on three manned islands but was done from a distance where species such as cormorants had dense breeding colonies. Other sites had at least weekly patrols that were in some cases done more frequently during the outbreaks.

A reporting form (Annex 4.1.3) for gathering morbidity and mortality data was prepared by L Roberts on behalf of provincial veterinary services and provided to managing authorities and rehabilitation centres. The form consisted of a Microsoft Excel spreadsheet, as well as a printable data collection sheet. Information requested for each observation comprised: date, place/ address, latitude and longitude in decimal degrees, affected species and numbers (in one column), status ("sick (alive)/ dead"), source of information, "Healthy birds of the same species", "Other species seen" (in an attempt to obtain the number of local susceptible birds), "other details" and test date and results (if applicable). The template was revised later to separate the columns for species and numbers affected. The completed form was submitted either to a supervisor who collated reports and sent them to L. Roberts at Veterinary Services or reports were submitted directly via email.

Some reports were not submitted in the requested format and sometimes this resulted in lost data, but often additional data were supplied. These additional data included age, what was done with the bird or carcass, whether the observation was made as part of a scheduled patrol, a reference number assigned to the bird, and sex determined on post-mortem examination at a rehabilitation centre.

In October 2021, a web-based reporting tool was built and launched, to aid reporting from the field and collation of data (https://survey123.arcgis.com/share/d5791b63ab65422ead648e64bff3238d). The tool was built by a DFFE Geographic Information Systems scientist, in consultation with L Roberts as a provincial veterinary epidemiologist, using the Esri ArcGIS Survey123 platform (Environmental Systems Research Institute, Inc., Redlands, California, USA). The data requested were very similar to the spreadsheet, but the location could be saved automatically and photographs attached. Reported data could be viewed online and downloaded via a Web Map (https://dea-surveys.maps.arcgis.com/apps/webappviewer/index.html?id=af4cb7f7ed4e4442ae861cfb60e128e6. Later, in August 2022, a dashboard with summary charts, tables and map was launched (https://dea-surveys.maps.arcgis.com/apps/dashboards/608a9c99f45c4be38bd7240cd853c113). Staff from conservation authorities, rehabilitation centres and municipalities received training via webinars in late October 2021 and were encouraged to use the tool.

A database of laboratory test results was maintained in parallel and cross-referenced with the field reports to avoid duplication and eliminate suspected cases that had tested negative.

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2.5. Data analysis

The number of confirmed cases, suspected cases, and negative tests, where available, are reported for each affected species. Negative tests performed at laboratories other than WCPVL in 2018 and tests at AssureCloud in 2021/22, that were not requested by WC Government or SANCCOB, are not available but are believed to be few. Relative case numbers at each location were also assessed and case numbers compared to colony size, where applicable (Department of Forestry, Fisheries & the Environment (DFFE) unpublished census data, 2022). African Penguin population size was calculated by multiplying the number of breeding pairs 2017 by 3.2 (Crawford and Boonstra, 1994). Locations within approximately 20 km of each other were grouped as one area. Where samples from multiple birds were pooled for testing, all birds were counted as negative if the pool tested negative but only one was counted where the pool tested positive. This conservative approach was taken because there was not always enough clinical information available to allow the assumption that all birds had been infected and not enough pooled samples to justify a statistical approach to prevalence estimation.

The challenges and lessons learnt during the response to the coastal bird HPAI outbreaks are discussed and analysed here. Records of decisions and experiences were gained from email and instant messaging records, minutes of meetings and workshops, and personal discussions.

3. Results

3.1. Confirmed and suspected cases

3.1.1. 2018 overview

Between December 2017 and May 2018, an estimated 7526 birds of 20 species putatively affected by H5N8 HPAI were reported from 31 sites along the western and southern coast of South Africa, in the Western and Eastern Cape provinces (Figure 3.1, Figure 3.2, Table 3.1). Most suspected cases were reported as having been found dead (n=7129), 199 are assumed to have shown clinical signs of HPAI (because they were reported as “sick”, “euthanised”, “alive”, or exhibiting neurological clinical signs or weakness with cloudy eyes), eleven were only reported as weak and for 193 birds, no status was recorded.

At least 209 suspected HPAI wild bird cases were tested, of which 115 tested positive, originating from 29 areas (Table 3.1). Negative results were only available from the Western Cape Provincial Veterinary Laboratory, so the true number tested is unknown but is unlikely to be significantly higher. Approximately 55% of positive cases were reported as having clinical signs of HPAI and about 30% were found dead. The remainder were simply weak and two were asymptomatic. These estimates are based on 105/116 positive birds with sufficient information.

Information on the type of sample collected for testing is available for 101/119 positive birds. Fifty-two were diagnosed on a single sample: 29 of these on a brain sample, eleven on pooled tracheal and cloacal swabs, seven on tracheal swabs, four on organ samples and the remainder on other combinations of pooled swabs. Of the 49 birds tested with more than one sample, a brain sample was tested for all but two birds (bringing the total brain samples to 76), and all but one of these brain samples were positive (a common tern with a positive tracheal and cloacal swab pool). Another 59 tests were done on the 49 birds (seven were tested on three or four samples), of which only ten tests were negative. These comprised four of seven cloacal swabs, two of twelve tracheal swabs and four of 34 cloacal and tracheal swab pools. One each of the negative tracheal and cloacal swab pools was pooled from the same four already-decomposing gannet carcasses.
A distinct, newly-introduced variant caused eight outbreaks in poultry in the northern part of the country during the same period. However, a Sacred Ibis was the only wild bird recorded to be affected

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(Abolnik, 2019) and the variant was not detected in seabirds or in provinces where affected seabirds were reported.

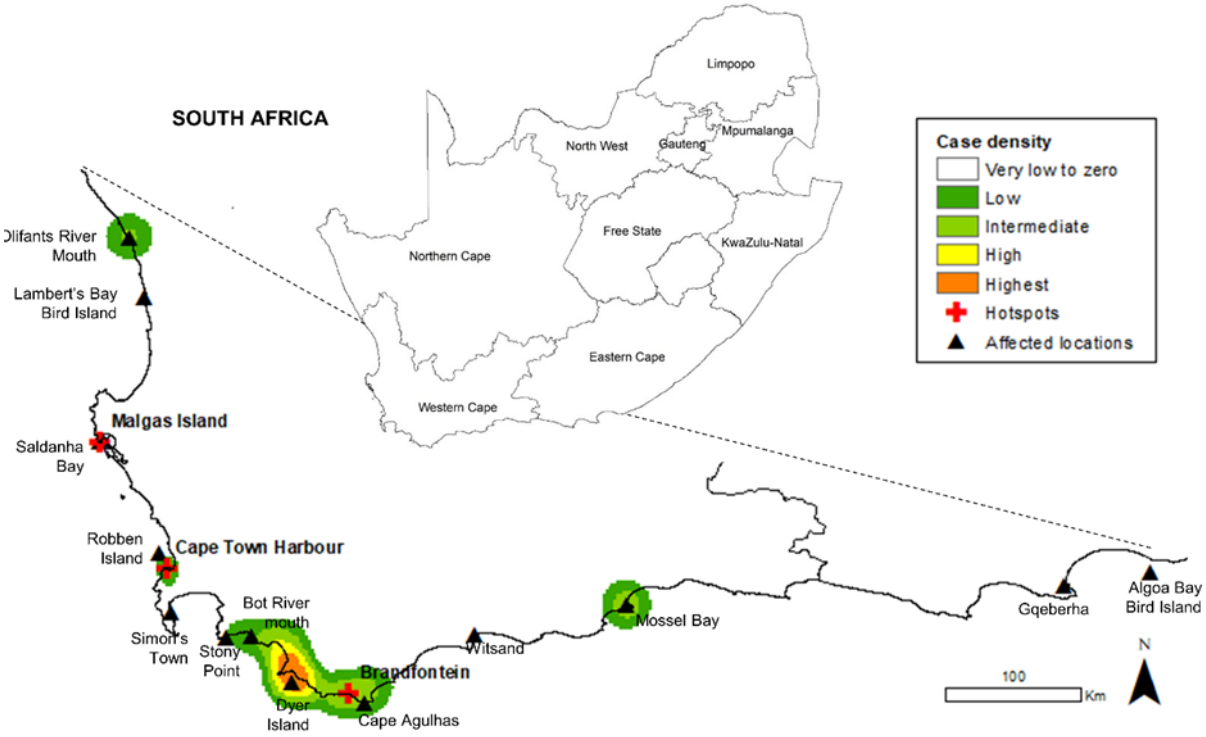


Figure 3.1: South Africa and its stretch of coastline from which high pathogenicity avian influenza (H5N8) in seabirds was reported between December 2017 and May 2018. The locations with more than 1000 dead seabirds are marked as hotspots. Malgas Island and the roof of a building in Cape Town harbour were the locations of outbreaks at Swift Tern colonies. The data from these hotspots were excluded from a kernel density estimation (KDE), performed using ArcMap software (v. 10.7.1, ESRI, Redland USA) with an output cell size of 2km km and a search radius of 30 km. The results of the KDE are shown as a heat map indicating relative case density.

3.1.2. 2021-2022 overview

Between 1 May 2021 and 31 December 2022, just under 25000 coastal birds were reported as affected by HPAI (H5N1), and counted as "suspected cases", from 22 sites in the Western Cape (Figure 3.3) and one in the Eastern Cape (Table 3.1). Approximately 24000 of these were Cape Cormorants, reported from October to December 2021, from Dyer Island and around Velddrif, on the West coast. Most cases in other species were also found in this period, but there was a significant, though smaller, peak in the mortality rate of African Penguins, on Dyer Island and in Simon's Town, from July to November 2022 (Figure 3.4). For larger groups, the records were not always clear about how many had been found alive and dead, but at least 19500 (78%) were found dead and at least 1 800 (7%) were euthanised.

Information on clinical signs was available for 854 birds found alive. Half had neurological signs, ranging from mild tremors or twitches to seizures, 20% (n=171) were only weak, 14% (121) appeared tame, 13% (107) were sick or stated to have shown signs indicative of HPAIV infection and the remaining 3% were reported to have a variety of signs less specific for HPAI.

At least 651 wild birds were tested for Influenza A during the 2021/22 HPAI (H5N1) outbreak. Not all negative results were available and 382 penguins were tested on admission to SANCCOB without necessarily showing clinical signs of HPAI or being suspected cases (see Chapter 4). HPAI virus was detected in 108 birds from 23 areas. Of 56 confirmed cases with data available, 42 (75%) showed some

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sort of neurological abnormality, 10 (18%) were simply tame, unresponsive, “calm” or “tired” and two (4%) only had drooping wings while two were simply underweight.

Sample type was available for 104 confirmed cases (96%). Fifty-two (50%) were diagnosed on a brain swab alone, 23 (22%) on pooled brain, oropharyngeal/tracheal and cloacal swabs, 23 (22%) on oropharyngeal/tracheal and cloacal swabs and one case each on an organ pool, on a brain swab and separate oropharyngeal/tracheal and cloacal swab pool (both tests positive), and a pool of “brain, trachea, other”.

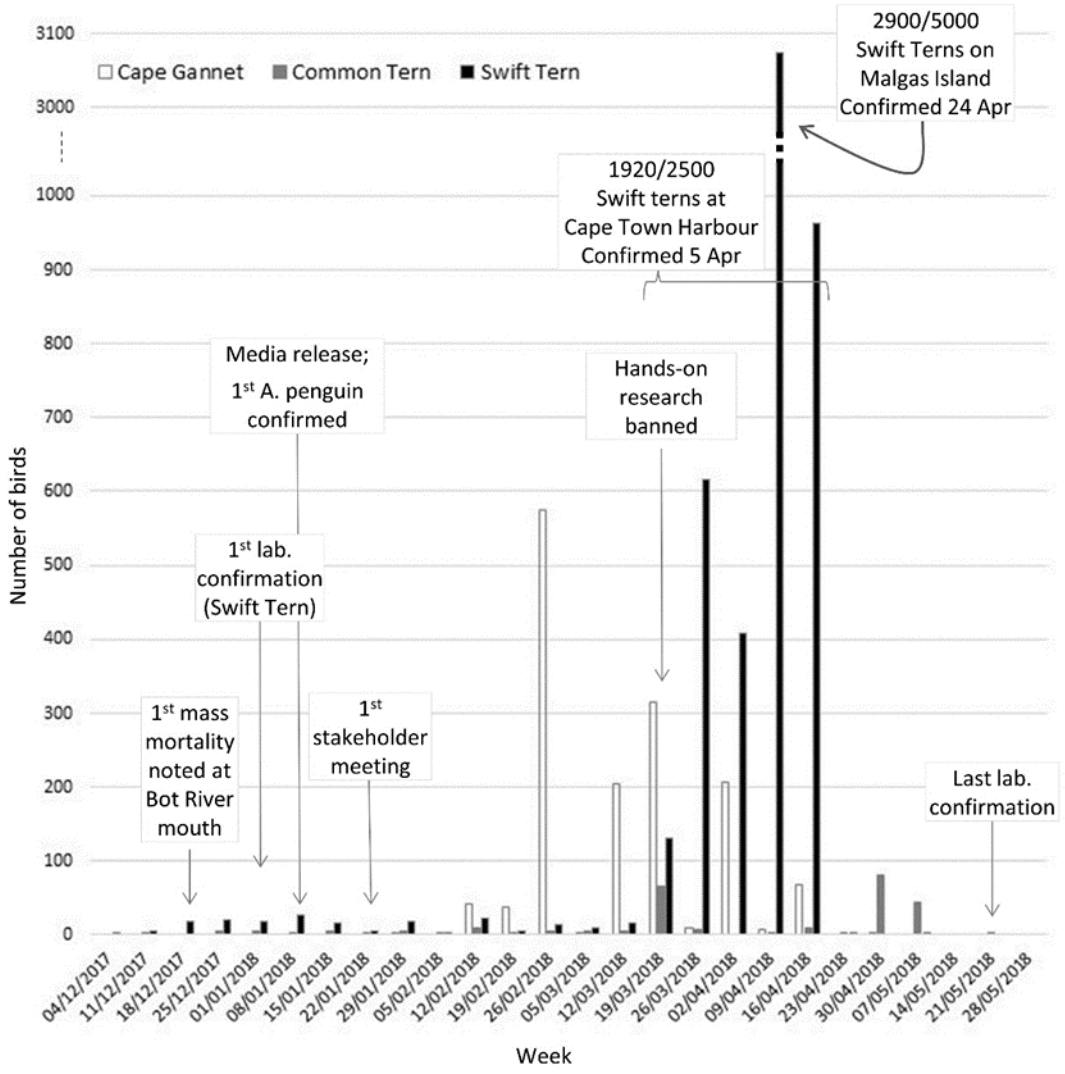


Figure 3.2: Weekly numbers of dead Cape Gannets, Common Terns and Swift Terns reported in South Africa during an outbreak of high pathogenicity avian influenza (H5N8), from December 2017 to May 2018. Dates of key observations, laboratory (lab.) confirmation, including the first confirmed African (A.) penguin case, and response events are indicated by arrows.

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Table 3.1: Laboratory-confirmed (pos.) and suspected (susp.) cases of clade 2.3.4.4.b high pathogenicity avian influenza in wild birds in Western and Eastern Cape provinces, South Africa (December 2017 to December 2022)

	2017/18					2021/22 (1 May to 31 Dec)				
Species	**First positive**	**Susp & Pos**	**Pos**	**Neg†**	**Pos areas**	**First positive**	**Susp &/ Pos**	**Pos**	**Neg**	**Pos areas**
African Oystercatcher *(Haematopus moquini*)	09 Mar 2018	5	1	2	1	26 Mar 2022	3	1	1	1
African Penguin (*Spheniscus demersus*)	12 Jan 2018	118	31	17	8	20 May 2021	334	37*	427**	5
Arctic Tern (*Sterna paradisaea*)	19 Dec 2017	10	0	0	0	-	-	-	-	-
Bank Cormorant (*Phalacrocorax neglectus*)	-	-	-	-	-	31 Oct 2021	6	4	2	2
Brown Skua (*Stercorarius antarcticus)*	-	-	-	-	-	15 Jun 2021	1	1	0	1
Cape Cormorant (*Phalacrocorax capensis*)	15 Feb 2018	104	5	15	3	06 Dec 2021	24 178	19	25	11
Cape Gannet (*Morus capensis)*	15 Feb 2018	1 473	2	20	1	13 Sep 2021	21	2	3	2
Common Tern (*Sterna hirundo*)	04 Jan 2018	288	8	1	8	17 Dec 2021	88	6	5	7
Crowned Cormorant (*Microcarbo coronatus*)	02 Mar 2018	2	1	0	1	(14 Oct 2021)	2	0	1	0
Egyptian Goose (*Alopochen aegypticus*)	16 Jan 2018	2	1	2	1	14 May 2021	9	2	3	2
Great White Pelican (*Pelecanus onocrotalus*)	-	-	-	-	-	28 May 2021	66	5	2	5
Great-winged Petrel (*Pterodroma macroptera*)	-	-	-	-	-	16 Oct 2021	2	1	3	1
Grey-headed Gull (*Larus cirrocephalus*)	23 Feb 2018	1	1	0	1	01 Jun 2021	1	1	2	1
Hartlaub's Gull (*Chroicocephalus hartlaubii*)	15 Feb 2018	24	8	32	4	17 May 2021	38	11	14	5
Jackal Buzzard (Buteo rufufuscus)	11 Jan 2018	1	1	0	1	-	-	-	-	-
Kelp (Cape) Gull (*Larus dominicanus)*	15 Nov 2017	17	0	16	0	20 May 2021	97	9	36	4
Northern Giant Petrel (*Macronectes halli*)	-	-	-	-	-	16 Oct 2021	2	1	0	1
Parasitic Jaeger (*Stercorarius parasiticus*)	02 Apr 2018	1	1	0	1	26 Mar 2022	2	2	0	2
Sabine's Gull (*Xema sabini)*	25 Feb 2018	1	0	1	0	-	-	-	-	-
Sacred ibis (*Threskiornis aethiopicus*)	30 Mar 2018	18	0	11	0	19 Jun 2021	12	2	2	2
Sandwich tern (*Thalasseus sandvicensis*)	27 Jan 2018	23	2	1	2	29 Nov 2021	5	0	2	1
Spotted Eagle-owl (*Bubo africanus*)	22 Feb 2018	1	1	0	1	-	-	-	-	-
Swift Tern (*Thalasseus bergii*)	20 Dec 2017	5 421	46	4	21	03 Mar 2022	56	2	10	2
White-breasted Cormorant (*Phalacrocorax lucidus*)	21 Feb 2018	16	0	0	0	11 Oct 2021	61	2	5	2
Yellow-billed duck (*Anas undulata*)	-	-	-	-	-	03 Jun 2021	1	1	0	1
TOTAL		**7 526**	**115**	**94**	**29**		**24 984**	**108**	**543**	**23**

† Western Cape Provincial Veterinary Laboratory rRT-PCR testing 1 Dec 2017 to 5 September 2018. The number of total tests done at other laboratories is not available.

* Includes 12 penguins and **370 penguins tested on admission to SANCCOB without a suspicion of HPAI necessarily (described in Chapter 4)

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3.1.3. Terns (Family Laridae)

Swift (Greater Crested) Terns (*Thalasseus bergii*) accounted for most suspected and confirmed cases (Table 3.1) in 2018. 5209 of 5421 cases , 5209 Swift Terns were found dead, 112 are assumed to have shown clinical signs, ten were only weak and 96 were of unknown status. Of the 50 known tests performed on the species, 46 proved positive and, of the sixteen positive swift terns with age recorded, ten were adults and six were juveniles. However, severe mortality (>60%) was observed among chicks and juveniles at two colonies on Malgas Island and at Cape Town harbour from mid-March to mid-April (Figure 3.1 and Figure 3.2), with fewer than twenty dead adults observed at the harbour. These events accounted for over 90% of suspected cases and only seven chicks and 82 juveniles were reported from elsewhere. Seventy-four juveniles were from Dyer Island (100 km south east of Cape Town), mostly in late March. A total of 167 carcasses were counted on Dyer Island, out of an estimated 4000 breeding pairs, so juveniles formed a lower proportion than elsewhere, and the mortality rate was far lower. On Robben Island, only one sick bird and one carcass tested positive in May 2018 out of approximately 2000 breeding pairs, and no increase in mortality was reported.

Admissions of sick Swift Terns to rehabilitation centres increased in early December 2017 (Figure 3.2). The rehabilitation centres saw birds that seemed neurological but only became suspicious as the cumulative number of cases grew. Avian influenza was not the first concern- the lack of cases while poultry was dying and the lull since October were enough to push AI further down the list of possible causes. Early discussion on social media about tern deaths speculated about red tides. The first noticeable mortality event involving three tern species (mostly Swift Terns) and two gull species, was observed on 18 December at Bot River mouth, de Mond Nature Reserve (Figure 3.1) (Khomenko *et al.*, 2018); however, the carcasses were too decomposed for AI virus testing and the deaths were attributed to nurdles (plastic pellets that are raw material for plastic manufacturing), found with the carcasses.

The first positive AI test in a seabird was from a Swift Tern found on 20 December 2017, at the same time as the first positive Swift Terns from Gqeberha (Port Elizabeth) in the Eastern Cape province, followed by regular reports of Swift Tern carcasses. The distribution covered most of the coastline from 200 km north of Cape Town on the west coast to Gqeberha in Algoa Bay, approximately 700 km to the east (Figure 3.2, Figure 3.5A). Clinical signs included initial weakness and inability to fly and “cloudy eyes”, likely due to corneal oedema. Affected birds then developed neurological signs, including head tremors, ataxia and circling, seizures, and death. Two of the positive chicks were apparently healthy, however. Two cases were reported 75 km inland, suggestive of severely impaired navigational ability because Swift Terns usually do not travel more than about 3 km from the coast (Hockey *et al.*, 2005).

Deaths in Common Terns (*Sterna hirundo)* in 2018 had a similar temporal and spatial distribution to Swift Terns (Figure 3.2, Figure 3.5B) but with lower morbidity and mortality. The largest single incident with Common Terns was 62 birds found dead in Mossel Bay, 330 km east of Cape Town in March. However, a larger die off (132 birds) 100 km north of Cape Town (Olifants River Estuary) between mid-April and mid-May 2018 was identified as Common Terns from photographs.

Only 56 Swift Terns were reported as suspected cases of HPAI (H5N1) in 2021/22 and most were from De Mond Nature Reserve (n=24) and Dyer Island (n=19) in October and November 2021 (Figure 3.4). However, the only two positive cases were from March 2022, during a period when few other cases were reported or detected. More Common Terns were reported as suspected cases (n=88) and there were also more virus detections in this species (n=6). Sixty-six percent (n=58) were reported dead from De Mond Nature Reserve from October to December 2021 and the rest were more evenly and widely spread. The positive cases were detected between December 2021 and August 2022, with two in May

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and June. One was a juvenile and age was not recorded for the other, but it was likely also a juvenile as some juveniles are known to overwinter (Hockey *et al.*, 2005).

Of interest was a dead sandwich tern (*Thalasseus sandvicensis*), positive on PCR for the H5N8 virus, found in Cape Town in January 2018, which was ringed in 2008 as a chick at Terschelling Oosterend, Friesland, Netherlands (SAFRING pers. comm).

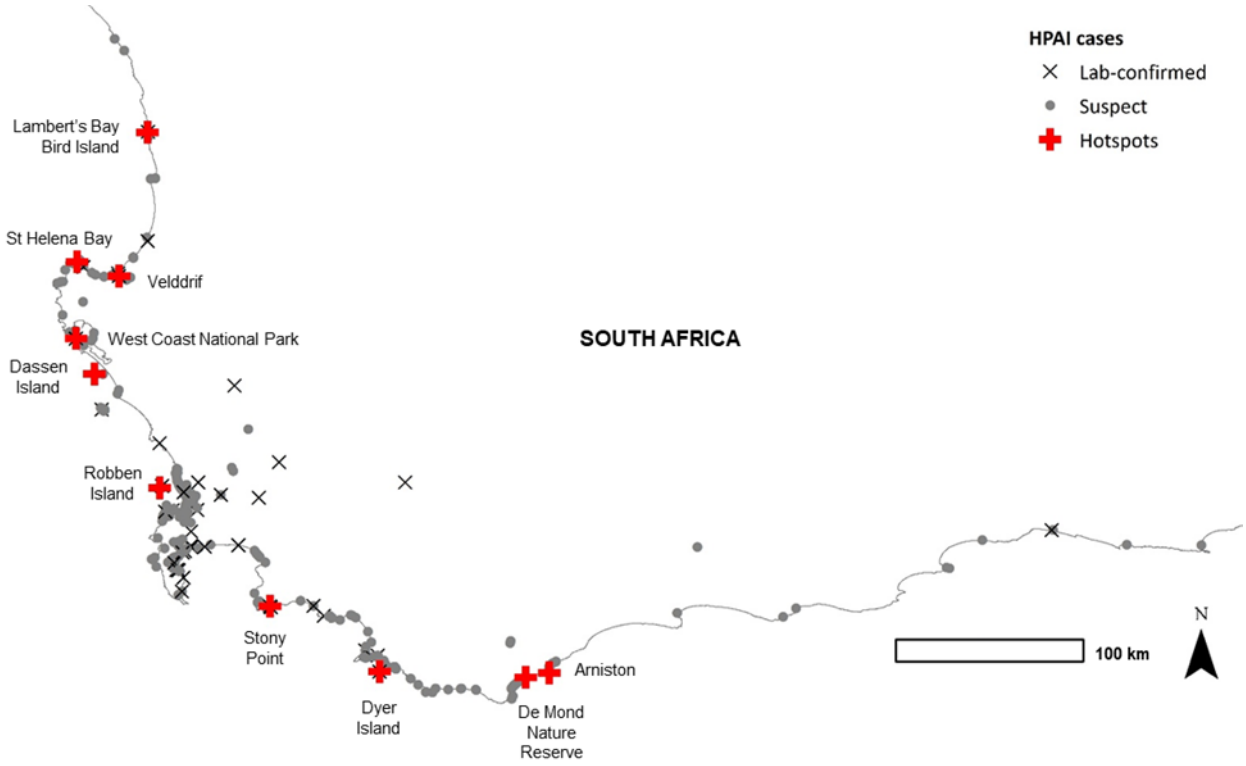


Figure 3.3: Locations from which clade 2.3.4.4b High pathogenicity avian influenza (H5N1) in seabirds was reported in South Africa between May 2021 and November 2022. The locations with more than 1000 dead seabirds are marked as hotspots.

3.1.4. African Penguin

Detection of avian influenza virus (AIV) in endangered African Penguins (*Spheniscus demersus*), caused significant concern, due to their Endangered status and rapidly-declining population, though fewer carcasses were found and proportionally fewer proved positive than Swift Terns (Table 3.1) in 2018 and Cape Cormorants (*Phalacrocorax capensis*) in 2021. The South African population was estimated at nearly 15 000 breeding pairs in 2018 (DFFE unpublished census data, 2022) equating to 48000 individuals (Crawford and Boonstra, 1994), so only 0.2% are counted here as suspected or positive 2018 cases. However, the population had decreased to an estimated 10000 breeding pairs (32000 individuals) in 2021, so the 334 suspected or positive cases from 2021 and 2022 represent a more concerning 1% of the population.

Ninety-nine of 118 suspected HPAI (H5N8) African Penguin cases (84%) were found dead. Of the 19 birds found alive, fourteen are assumed to have had clinical signs, one only had difficulty breathing and four were of unknown status. One penguin was found with only a head injury, but developed neurological signs and tested AIV PCR-positive. Of the 92 suspected penguin cases where age was recorded, 75 were adults, 12 were juveniles and five were chicks. Of the 25 confirmed cases with age data, 22 were adults and three were juveniles. Sex data were available for seventeen positive birds examined post-mortem: thirteen (76%) were females.

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The first infected African Penguin was reported on 12 January 2018 (Figure 3.2), approximately 100 km east of Cape Town, and 10km km east of Bot River mouth. Cases were detected between Cape Town and Gqeberha until May 2018 (Figure 3.5C). Fifty dead or moribund penguins were detected in the False Bay area, southeast of Cape Town, including nineteen birds from the Simon's Town colony (five confirmed cases), between 29 January and 30 March, and eight from the Stony Point colony (five confirmed) in April and May. Four positive and eleven suspected cases were reported from Dyer Island, further east, between February and May and three penguins from Robben Island, north of Cape Town, tested PCR- positive in May. Ten suspect and one positive case were reported from Algoa Bay (Gqeberha). Clinical signs in penguins included mucoid ocular discharge, cloudy eyes, apparent blindness, lethargy and an inability to stand, open-mouthed breathing, and neurological signs such as head or whole-body tremors, head tilt, dorsal neck flexion, bilateral nystagmus and seizures. A penguin with mild neurological signs was treated supportively and with 35mg oseltamivir twice a day, under isolation, for 3 weeks. Although a PCR test after two weeks of treatment indicated that virus shedding may have ceased, the bird was euthanized due to a deteriorating neurological state. A post-mortem PCR test of the brain was positive for H5N8 virus. Incidentally, a serum sample taken before euthanasia was also tested for avian influenza antibodies, using the methods for haemagglutination inhibition (HI) described in Chapter 5. Positive titres (≥1:16) were detected for all H5 antigens used (H5N8 1:512, H5N1 1:16, H5N2 1:32) and H6N8 (1:64), as is expected for antibodies to a clade 2.3.4.4b H5N8 infection (SANCCOB unpublished data, 2022).

In the second epizootic, the first African Penguin tested positive for HPAI (H5N1) in May 2021 and originated from Simon's Town. However, 210/232 (91%) of suspected and confirmed African Penguin H5N1 cases from the 2021 outbreak and 63% overall were reported from Dyer Island between October 2021 and January 2022 (Figure 3.4). One hundred and ninety-six (196) were reported as having been found dead but few other details (such as age) were recorded. There were eight positive tests on penguins from Dyer Island during this time. Three tests were on swab pools of two penguins each (two pools with adults and one with a chick and a juvenile) so eleven penguins were swabbed, in total. Of these, three were chicks, one was a juvenile and seven were adults, and of seven with sex recorded, four were male.

Another fifteen penguins were reported from Dyer Island the next year, in July and August 2022, and HPAI H5N1 was detected in a swab pool from two birds. All were adults except one juvenile. Approximately half were found dead and the others had neurological signs.

Most of the rest of the suspected or positive H5N1 African Penguin cases (19%; n=62) originated from Simon's Town from August to November 2022 (Figure 3.4) and 22/38 (58%) of confirmed African Penguin H5N1 cases were from this period and site. More specifically, 55/62 suspected cases and 19/38 positive tests were from Foxy Beach, in the Boulders reserve. Of the positives, 15 were adults, three juveniles and one chick and, of the 18 with sex recorded, eleven were male. Of 47 suspected cases from Foxy Beach with age recorded, 42 were adults, three juveniles and two chicks.

Of the 55 confirmed and suspected cases from Foxy Beach, 39 (71 %) were found dead. Clinical signs were recorded for eight of those found alive and seven tested positive. Neurological signs were seen in six of the positives and the last was unresponsive. One tested negative but showed mild neurological signs, so was counted as a suspected case.

In October 2022, 63 African Penguins died at Boulders after being stung by a swarm of bees. Before the cause was identified, eighteen were sampled for avian influenza testing and three tested PCR-positive. HPAI infection was not suspected based on post-mortem examination.

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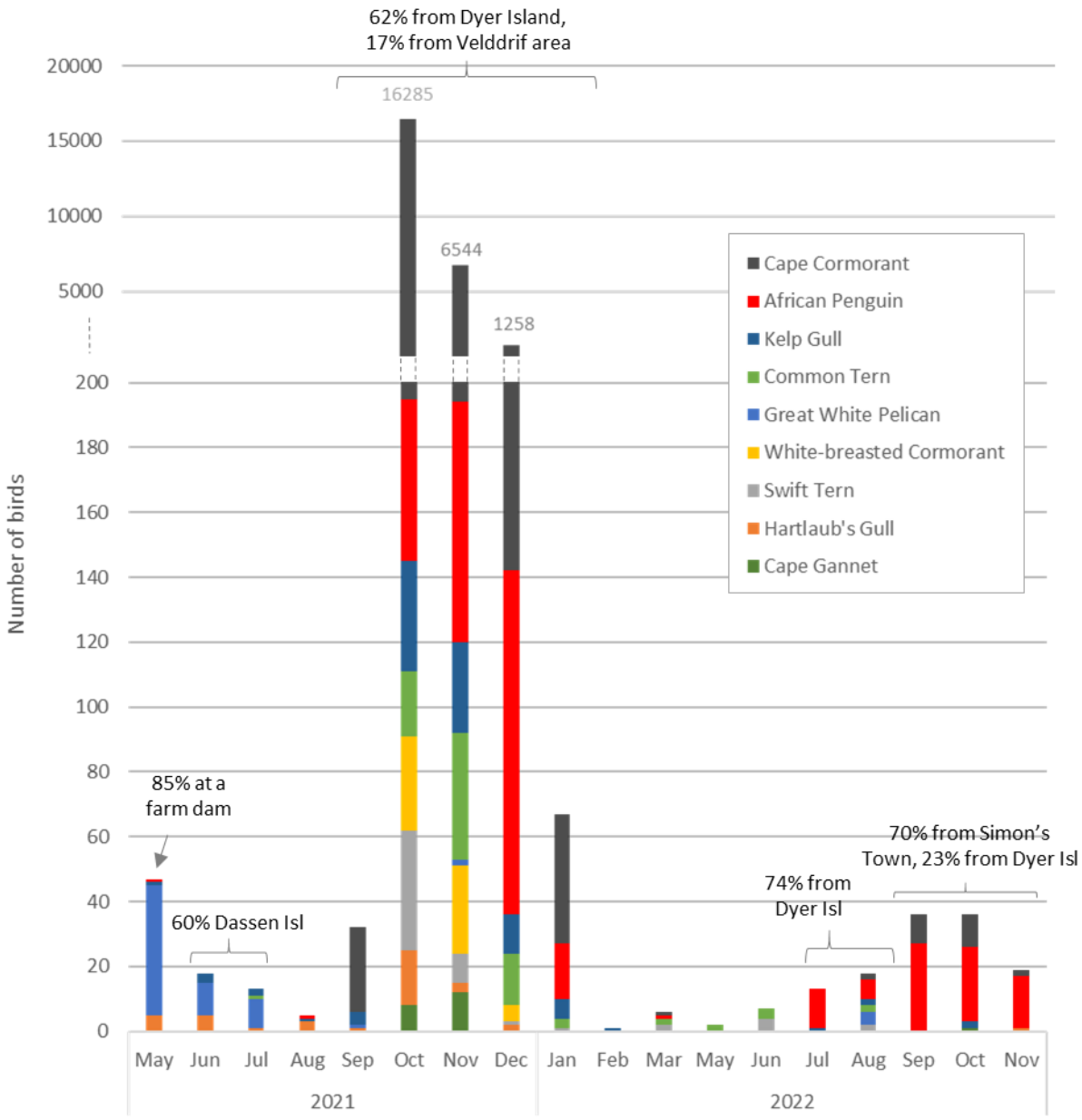


Figure 3.4: Monthly numbers of seabirds suspected to be affected by an outbreak of high pathogenicity avian influenza (H5N1) in South Africa from May 2021 to November 2022. The nine species with the largest numbers of cases, representing 99% of the total, are shown and are listed in the key in order from largest to smallest number of cases. For each peak in cases, the sites responsible for most cases are indicated.

3.1.5. Cape Gannet

In 2018, only two of 22 Cape Gannet samples tested AIV-positive, although approximately 1500 Cape Gannet (*Morus capensis*) carcasses were found between February and April 2018 on beaches west of Cape Agulhas, the southernmost tip of Africa (Figure 3.2, 5D). The age was recorded for half of these birds: 40 were recorded as juveniles and the remainder as adults. However, there was no corresponding increase in mortality in the three South African breeding colonies and only two pooled samples tested positive for AIV while at least another twenty tests were negative (Table 3.1). Additionally, 60 healthy birds sampled at the Malgas Island breeding colony in February tested negative and no clinically-affected birds were recorded. Other seabird carcasses were found near Cape Agulhas in much smaller numbers, and carcasses overall were in various stages of decomposition, with signs of substantial scavenging and likely predation, possibly by seals. Smaller numbers of gannet

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carcasses were found at later surveys in November 2018 and January and March 2019 and were attributed to seal predation.

Small numbers of Cape Gannets were suspected and confirmed to be infected in 2021 and 2022. Most suspected cases (18/21) came from October and November 2021 (Figure 3.4), from the West Coast. Eleven were found at the Lambert's Bay breeding site and six in West Coast National Park, which includes Malgas Island, another breeding site. The two birds that contributed to the pool that tested positive in 2021 were tame and weak, with mild head twitches. The single, confirmed case in 2022 also originated on Malgas Island.

3.1.6. Cape Cormorants (Family Phalacrocoracidae)

In 2018, most suspected and confirmed cases in Cape Cormorants (*Phalacrocorax capensis*) were reported from the south coast, east of Cape Agulhas (Figure 3.5E). Sixty of 104 Cape Cormorants were reportedly found dead. Of the 44 live birds, 24 displayed neurological signs and these were the source of five out of six confirmed cases (Table 3.1). A large number (n=180) of juvenile, emaciated Cape Cormorants without neurological signs were found in and around Cape Town and were admitted for rehabilitation in the same period (SANCCOB unpublished data, 2022). However, the number admitted is not unusual and these were not tested nor counted as suspected cases.

Cape Cormorants accounted for 97% of deaths due to HPAI H5N1 in 2021 and 2022 (n=24177), and the affected number equates to approximately 30% of breeding adults counted in 2020 (DFFE, unpublished census data, 2022). Dyer Island was the site of 62% of these Cape Cormorant deaths, while 18% occurred in the St Helena Bay/ Velddrif area on the West Coast and over a thousand carcasses (4%) were found in West Coast National Park (Jutten Island), De Mond Nature Reserve (Heuningnes Estuary) and Stony Point Nature Reserve. The majority were found from October to December 2021 (Figure 3.4) and at least 20 000 were found dead, while most of the rest were euthanised. A small number could not be caught. Only one coastal bird case is known to have been confirmed outside the Western Cape; a Cape Cormorant from Cape St Francis, in the Eastern Cape. Information on this case was sourced from the World Animal Health Information System (WAHIS).

Nineteen positive tests came from Cape Cormorants. The Dyer Island outbreak was confirmed on one test on samples pooled from four Cape Cormorants (two males and two females) that showed hepatomegaly and hepatic petechiation and haemorrhage. Three cases from Velddrif were confirmed, three from Stony Point and one from Jutten Island but none from De Mond Nature Reserve. Of the fourteen confirmed cases with age recorded, eleven were adults and three were juveniles. Nine out of fourteen with known sex were females. Of the eleven found alive, with records of clinical signs, six showed obvious neurological signs such as head twitching and torticollis, four were abnormally tame, two unresponsive and two had a swollen head.

Sixteen suspected HPAI cases were reported in White-breasted Cormorants in 2018, though none were tested. In contrast, 61 suspected cases were recorded in the species in 2021 and two of the at least seven samples tested were positive. All 2021 cases were reported between October and December (Figure 3.4). Thirty were from Dyer Island and 14 were from De Mond Nature Reserve. The two confirmed cases were from Gansbaai and Cape Town, in October 2021.

Bank Cormorant cases were not recorded in 2018 but four confirmed cases and two suspect cases were reported in 2021, from October to December. Three were found in Sea Point and one at Clifton, nearby. Two confirmed cases were adults, found dead, and two were juveniles, that were both found alive.

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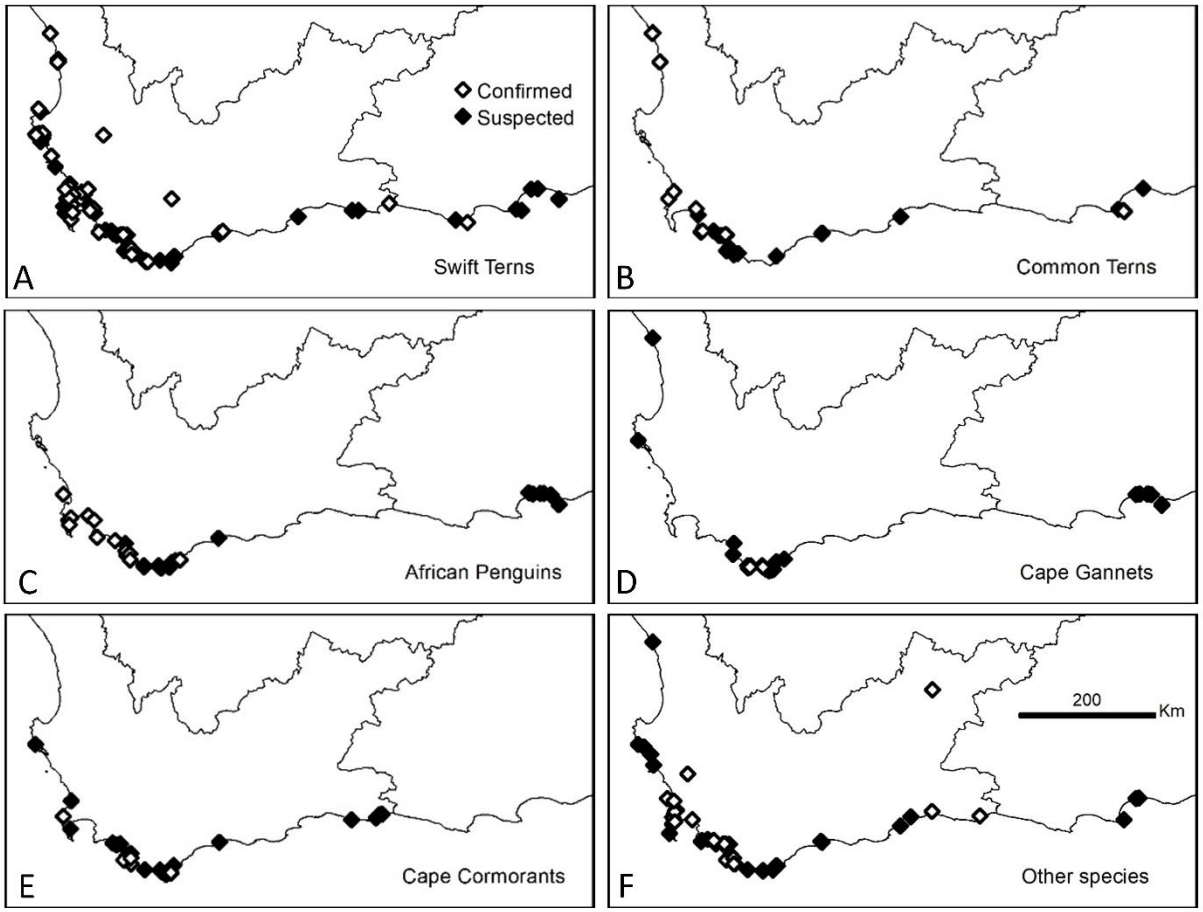


Figure 3.5: Locations along the South African coastline of different species of seabirds that were suspected and confirmed (via rRT-PCR) to have died from high pathogenicity avian influenza (H5N8) between December 2017 and May 2018.

3.1.7. Other species

Suspected and confirmed cases in other species, reported in smaller numbers, are described in Table 1 and Figure 3.5F.

Gull carcasses were reported regularly, starting in January 2018, but not in large numbers. One Hartlaub's Gull (of six tested) and one Grey-headed Gull tested positive in February 2018. However, five of six Hartlaub's Gulls found around Cape Town tested positive in April, and another positive was found at Blouberg beach on 11 May. A few individual Kelp Gulls and some from a batch of fourteen dead and dying on a beach, with eleven dead Hartlaub's Gulls, near Saldanha Bay in early March 2018 tested negative but none were positive. One Kelp Gull recovered after treatment with activated charcoal.

Hartlaub's Gulls were the first seabirds to test HPAI H5N1 positive in May 2021 and a Kelp Gull case was confirmed days afterwards. Detections in both species continued until August 2021 and there were three more in January, February and October 2022. Eight of 11 confirmed Hartlaub's Gull cases were adults and three juveniles. Seven of 12 were male (all three juveniles included) and seven were found alive. Clinical records were available for five of these and all showed neurological signs. Seventeen of the 38 suspected and confirmed cases were reported in October 2021 and the site with the most (n=6) was Velddrif.

Kelp Gulls were the species with the fourth-highest number of suspected and confirmed cases reported (n=97), mostly from October to December 2021 (Figure 3.4), though 9 of approximately 45

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tests were positive. Twenty were from De Mond Nature Reserve in late 2021 but the 20 from the West Coast, just north of Cape Town, were recorded between May 2021 and October 2022, though with a peak late in that period. The 14 cases on Dyer Island were reported between October 2021 and August 2022 and the cases from Simon's Town and the northwest area of False Bay between August 2021 and October 2022. Eight of nine positive Kelp Gulls had sex and age recorded. Three were adults and five were juveniles, while four were female. Six were found alive and four of these had obvious neurological signs. Two showed drooping of one or both wings, and no other signs, and one with neurological signs also displayed corneal oedema.

Forty of the 66 reported suspected Great White Pelican cases were carcasses from a farm inland, in May 2021 (Figure 3.4). An H5 PCR test was positive on one bird from this group. Twenty-four suspected cases were also reported from Dassen Island, 18 in June and July 2021 (Figure 3.4), and the H5 virus was detected in one bird. Four pelicans were found alive on Dassen Island and were seen walking in circles, ataxic or simply weak. A positive case from a landfill site had intention tremors and apparent confusion, docility and unresponsiveness and another found at Cape Town International Airport showed only a hanging head and shaking wings.

3.2. Outbreak Response

3.2.1. Communication

The national veterinary authorities were first notified of the detection of HPAI (H5N8) in Swift Terns on 5 January 2018, the day that laboratory confirmation was received by provincial veterinary authorities, and twenty days after the first positive samples were taken. The public was informed four days later, via a media release (Figure 3.2; Western Cape Government, 2018). A contact list of stakeholders was developed as a priority to provide information and advice on outbreak management.

The Western Cape provincial veterinary services provided general advice on outbreak response and reporting, while rehabilitation centres issued more specific guidelines related to their own functioning, including admission of sick and dead birds. Summaries of information with specific guidelines were distributed among conservation staff members (Kock, 2018). The first meeting with stakeholders was held in mid- February after the virus was detected in penguins (Figure 3.2). Monthly meetings occurred between the leading role-players (Government veterinary and environmental agencies and conservation authorities) to adapt response activities and report progress.
Regular media statements were released to keep the public informed and to advise on handling dead and sick birds. These prompted queries from journalists, especially after the news that infection was detected at the mainland penguin colony in Simon's Town, near Cape Town, a major tourist attraction (South African National Parks, 2018).

Western Cape Veterinary Services, the provincial Disaster Management Centre and provincial waste management licencing officials had worked closely during poultry HPAI outbreaks in 2017. When large-scale mortalities in seabirds were detected on the west coast in October 2021, district and then provincial disaster management centres were approached to assist with coordination of the response and communication with waste management authorities about disposal methods. This contributed to a more structured and better-coordinated response than in 2018. Regular online meetings (every two to five days in October and then weekly in November) were held to update stakeholders, discuss challenges and also to assess the need for updates to the public. Media updates were facilitated by a joint ministerial spokesperson for both Disaster Management and provincial Environmental Affairs, who consulted with spokespeople and technical staff from the Department of Agriculture.

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3.2.2. Outbreak monitoring and reporting

The initial media release in January 2018 requested the public to report suspected cases to their local state veterinary or government office. An official request for two-weekly reports, citing relevant legislation, was issued in early March to government veterinary offices, relevant conservation authorities and rehabilitation facilities. The standardized template for reporting observations was supplied.

Four hundred and twenty-seven (427) reports (each related to a single site and date) were received by Western Cape Animal Health in 2018: approximately 55% from rehabilitation centres, mostly from reports by the public, 20% from the provincial conservation authority (Cape Nature), 11% from South African National Parks (but 40% of suspected cases), and the rest from other sources such as BirdLife South Africa, the public, municipal staff, private vets, Cape Town aquarium staff (reporting 25% of suspected cases) and the local Society for the Prevention of Cruelty to Animals (SPCA). Of 40 reports from the provincial authority, twelve were scheduled patrols and 28 “ad hoc observations”. Forty-nine reports were excluded; because they related to birds that later tested negative (four reports), with unspecified numbers (“lots”, “several”), where species was not determined (e.g., “Tern”: 27 reports, 169 birds, “Cormorant” (two reports), no species (two reports) or related to species with only single reports or a small number of birds found dead and with no laboratory confirmation in the species (14 reports). Data were received from rehabilitation centres and conservation authorities after formatting and cleaning, so were generally of good quality and easily collated by that stage. Some problems encountered included missing geographic coordinates, no location stated at all, or location names without coordinates, that could not be traced. The columns requesting denominator data (healthy birds of the same species and birds of other species) were never completed, though this information was sometimes obtained through further questioning (e.g. for estimated numbers of susceptible Swift Tern chicks in 2018). Another challenge was duplication of reports, when the conservation authority sent birds to a rehabilitation centre and both organisations reported them. This was particularly difficult to resolve if different dates were recorded, for example, the date found and the date of arrival at the rehabilitation centre. Although several private veterinary practices assisted with the euthanasia of affected birds, very few cases were officially reported from these practices so it is likely that this resulted in some under-reporting.

The data received were first published as an interactive Google map, accessible online to anyone with the web address, at the end of March 2018 and then as a status update in May, amended in September.

Of 2 473 reports received in 2021 and 2022, some of them reporting null sightings, 1 757 (71%) were from CapeNature, the Western Cape conservation authority, and these reports covered 82% of suspected and confirmed cases. Most reports were based on patrols scheduled between October 2021 and January 2023, when cases peaked. Three hundred and three reports (12%) were from the SANCCOB rehabilitation centre (1% of cases) and another 40 reports (2% of reports, 38 cases) were from their field rangers stationed on Robben Island and at Stony Point. Municipalities provided 7% of reports (n=173, 2% of cases), mostly from staff dedicated to conservation work. The remaining approximately 8% of reports came from other animal rehabilitation centres, the national conservation authority (SANParks), the provincial Department of Agriculture, SPCA, members of the public, BirdLife South Africa and a stranding network. However, a bird rehabilitation centre from the west coast reported 7% of cases in 20 reports and SANParks reported 6% of cases in 30 reports.

The first case reported via the online application was observed on 13 October 2021. A total of 468 entries, reporting 251 suspected cases, were made in 2021 and 2022. CapeNature did not use the app in 2021 as staff were already reporting reliably via the spreadsheet. However, in 2022 the data were sourced either from the app or directly from SANCCOB, with few, if any, reports via other sources.

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The main users of the app were a City of Cape Town official (131 reports; 28%), SANCCOB (102; 22%), an Overberg District Municipality official (76; 16%) and DICT (65; 14%) and the remainder from five other organisations and an "other" category.

Reports from CapeNature were received daily, from October to December 2021, and data were cleaned and collated weekly. Summaries were presented as tables and epidemic curves in meetings or distributed as a report to stakeholders, via email and instant messaging services. A Google map link was also provided.

3.2.3. Response in the wild

The goals of managing the disease in the wild were to minimize viral spread and stress to wild populations. Management was limited to removing carcasses and sick birds as sources of the virus, where possible, and limiting the additional mechanical spread of the virus and disturbance caused by human activities.

Activity in seabird colonies was minimized by banning all hands-on research activities in late March 2018 (Department of Environmental Affairs, 2018; Wild, 2018) and restricting monitoring activities to remote methods (Figure 3.2). In June, after the first penguin breeding peak and a month since the last confirmed case in a coastal bird, it was decided that low-impact, non-invasive research activities would be allowed, with biosecurity protocols to be followed. The protocol provided basic guidelines for the use of disinfectants, including the principles of correct active ingredient, concentration and contact time and the negative effects of excessive biological material. Recommendations included waterproof clothing or a plastic apron, rubber boots and gloves. The waterproof clothing proved extremely uncomfortable in hot weather. In September, more intrusive procedures and guano sampling were permitted to proceed. The insertion of passive integrated transponders (microchips), used to identify and monitor individual birds, was only allowed again in early 2019.

In 2021 and 2022, there was no blanket research ban, and research continued at many sites, with biosecurity protocols agreed to. However, some colony managers took a precautionary approach and did not allow research for periods when there were higher numbers of cases in their colony or elsewhere.

The public was requested via media releases to avoid handling dead bird carcasses, especially if they had contact with domestic birds. At the seabird colonies, conservation authorities distributed information to staff, on the disease and instructions for biosecurity and managing sick birds and carcasses and record-keeping. Protective clothing, including gloves as a minimum, rubber boots, disposable aprons, and face masks for added protection was to be worn in the colonies, especially when handling sick birds and carcasses, and disinfectant was distributed for application to equipment, clothing, footwear and vehicles. Compliance was high among the staff of management authorities, especially at mainland colonies, and at rehabilitation centres, possibly due to some fear of personal infection. At the two mainland penguin colonies, additional measures were required to manage visitors, including footbaths or restricting access to the raised boardwalks. Notices were also displayed to inform guests of the situation and potential risk to domestic birds. Over the peak outbreak period, the release of African Penguins and other seabirds from rehabilitation centres was discontinued at the Simon's Town African Penguin colony.

Information about avian influenza in humans was distributed to conservation authorities' staff, with instructions to visit the nearest clinic if flu-like symptoms were experienced. However, it was emphasised that there was no evidence that the HPAI (H5N8) virus had caused any mammal or human infections. The Department of Health was informed of the locations of the important bird sites and distributed instructions and sampling equipment to the closest clinics by early April.

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Carcass disposal was attempted where possible or necessary in public areas. Guidelines issued by provincial veterinary services in April 2018, and again in October 2021, listed disposal options and provided contact details for waste management and air quality control officials and carcass disposal experts. It was suggested that carcass masses of less than 10 kg be buried at least 1 m deep, above the high tide mark. This was given special approval by provincial waste management authorities, since routine burying of infected carcasses is illegal under national waste management legislation. The Swift Terns from Cape Town harbour were incinerated, which accounted for approximately 25% of carcasses in 2018. Swift Tern carcasses from Malgas Island comprised approximately 40% of 2018 carcasses and were either buried on the island or burnt in pyres. Another 13% were buried on the beach and disposal method is unknown for 18% of suspected cases reported in 2018. The majority of Cape Cormorant and penguin carcasses counted in 2021 were cleared daily and buried. The burial sites were approved by the waste management and municipal authorities as part of emergency measures. The site for Dyer Island was on the mainland, at a site chosen to be as close as possible to the harbour, and the process involved transport of carcasses in plastic bags, via boat. Those carcasses that were removed were collected by hand, by staff wearing protective clothing that included gloves, overalls and boots and masks when carcasses were burnt. A disinfectant footbath and spray were also used at the harbour. Some would have been submitted to a rehabilitation centre and therefore would have been incinerated. These were transported in a triple layer of plastic bags. It is expected that some were also left on the beach.

Euthanasia was advised for Swift Terns and other species of low conservation concern with suspicious clinical signs, given that treatment had already proven unsuccessful. Private veterinarians, without frequent bird patients, assisted with chemical euthanasia (with some days spent on-site in Velddrif) and training on cervical dislocation was given to field staff by the SANCCOB vet in late 2021. Penguins and Bank Cormorants with mild or moderate signs were accepted for assessment at the rehabilitation centres, given their endangered status.

3.2.4. Management in seabird rehabilitation centres

The two main seabird centres were advised mainly by in-house or contracted veterinarians. One shared their information sheet and protocol with smaller centres handling a wider range of species, conservation authorities and veterinary practices. This covered the collection of suspected cases, transport to and handling at the centre, equipment needed, isolation procedures, waste disposal, reporting requirements and sample collection.

The provincial veterinary authorities considered quarantine of seabird rehabilitation centres in the Western Cape in 2018 but did not implement it. Preventing admission of new patients to rehabilitation centres was deemed unreasonable, given their function. Though pre-release testing was done at SANCCOB, the largest seabird centre, between mid-February and the end of March, no positive results were obtained, and it was discontinued. Increased biosecurity measures were instituted to protect the rehabilitation centres. These included the use of footbaths, improved disinfection, isolation facilities for suspected AI cases and the euthanasia of severely affected cases without admission to the rehabilitation centre. During the peaks of the outbreaks, all birds with any neurological signs were euthanized and not admitted to the rehabilitation centre. The question of pen-side avian influenza tests was raised a few times, when considering methods of screening birds before admission to rehabilitation centres. However, their use is not permitted in South Africa, as testing for controlled diseases must be done in approved laboratories, and there were concerns about the tests' sensitivity and value as screening tests.

In late 2021 and again in late 2022, SANCCOB set up another facility, in a warehouse, to hold birds, mainly chicks, from infected colonies or with suspicious clinical signs. They were observed for a minimum of 14 days and, where funding allowed, a representative sample was tested with PCR. Each

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week's admissions were assigned to one group and the 14 days were counted from the date the last bird was added. They were moved to the main facility once a low risk of avian influenza had been established. Each group was separated by at least 5 meters and separate protective clothing and equipment were used. Where possible, separate staff were assigned to each group.

Available records from SANCCOB reflect that birds euthanised specifically on suspicion of HPAI, from January to May 2018, included 51 Swift Terns, of which twelve were tested and one was negative, ten Common Terns (one was tested and positive), five penguins (one tested negative, a chick), and two Cape Cormorants (one tested negative, the other was not tested). These data may not be completely accurate because record keeping was difficult and many more already-dead birds were handled, but stricter record-keeping protocols were instituted as the outbreak progressed. Treatment was attempted in a few penguin cases, but they usually died within 12 hours of admission or were euthanized to prevent further suffering. In comparison, in the same period, the centre released 165 African Penguins, 154 gulls, 34 Cape Cormorants, one Cape Gannet, zero Swift or Common Terns and eight birds of other species.

3.2.4.1. HPAI outbreaks in a rehabilitation centre

In late June 2021, two 4- and 6-week-old Hartlaub's Gull chicks that hatched at SANCCOB tested positive for avian influenza. One was euthanised after showing neurological signs and the other died acutely. The eggs were found in the wild and though vertical transmission may be possible, the disease manifestation at that advanced age makes it seem unlikely. They must therefore have been infected within the centre, but the source was not clear. They had been in the same room as a Kelp Gull that tested positive after dying in the centre. However, at least two other Kelp Gulls tested positive from within the centre, and it is not possible to tell whether they were infected when they were admitted or became infected afterwards. They were admitted for suspected botulism, with signs of weakness, and one was also wounded. One was euthanised after showing neurological signs and the other when it failed to recover from the apparent botulism. No further evidence of disease spread was detected. Birds with suspicious clinical signs tested negative and some birds tested pre-release were negative.

Another and more extensive outbreak occurred at SANCCOB in late 2022, when SANCCOB had just over 400 African Penguins in care, mostly chicks. On 21 November, an abnormal number of penguins were observed with signs of corneal oedema. Some corneal oedema or milkiness of an eye is normal due to trauma from foreign bodies in the colony, or and scratches from other penguins, but the number became unusually high. The next day, one fledgling had a head tilt and inflamed eyes and was swimming in circles and into walls and having seizures. It died overnight and tested positive for H5 AIV post- mortem. It had been admitted to SANCCOB a month before as an underweight fledgling and was in the group being prepared for the weekly release.

The source of the virus was not clear. Before 15 November, all birds admitted from Simon's Town, which has been experiencing an HPAI outbreak since August, were first quarantined at the offsite facility and a representative sample was tested. The facility was closed on 15 November, however, as it was empty and most chicks had been cleared from Simon's Town. In the week preceding 21 November, relatively large batches of penguin chicks were received from Stony Point and one or two arrived from Simon's Town. None of these showed signs of HPAI but a carcass from Stony Point had tested H5-positive in early November. A weak Cape Gannet was admitted to SANCCOB on 19 October, from Malgas Island. Its tail was pulling to one side, it was ataxic and spinal damage was suspected. However, mild corneal oedema was noticed on 2 November, after 13 days in the rehabilitation centre and it was euthanised. It tested positive for H5 avian influenza on PCR samples collected post-mortem, though the laboratory results were only received on 14 November. The three- week gap between the gannet's euthanasia and the first clinical signs in penguins implies a rather long incubation period. However, it's possible that early cases in penguins were missed.

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Further sampling, to confirm the disease and its spread, was done on 23 November. Penguins with clinical signs and those in the pen from which the fledgling had been removed were treated as one epidemiological group and the adjacent pens were treated as a second group. A total of 60 birds were sampled with tracheal and cloacal swabs and samples from 10 birds were pooled for each test. Six of eight pools tested positive, indicating that at least four of five pens were affected.

By 29 November, four more penguins had displayed neurological signs and had been euthanised and another 26 had developed corneal oedema, but no other signs. Birds with clinical signs had been found in most of the 12 rehabilitation pens but the approximately 35 resident "Home Pen" penguins remained healthy.

Given the decreased incidence of cases reported from the colonies and the high prevalence in the centre, the state veterinarian decided to quarantine the facility. The centre was closed to visitors and birds requiring treatment were admitted to the reopened offsite facility. Only severely ill or injured birds could be admitted to the main facility. Foot baths were placed at the main entrance, and entrances to all pens and rooms holding birds, and staff were assigned to different sections.

Initially, any penguin that showed clinical signs (mostly corneal oedema) was immediately removed and placed in an isolation room. This was stopped after almost all birds in the centre were sampled on 12 December and tested, with pooled swabs. The isolated group, which had been sampled the week before (one of ten pools positive), and the Home Pen birds were excluded. Virus prevalence was estimated at 4% (95% CI 2.6-6.9%) and the only non-penguin infected was a Cape Gannet, although it was in a pen with other species. All penguin pens were affected, except for one section, which contained the “medium-sized” chicks; large enough to have graduated from the “chick-rearing unit” but too young to be prepared for release. They were kept in a room separate from the pens with older penguins, with dedicated staff and equipment and no direct contact with other birds. Most other pens had through-the-fence contact. In total, 329 penguins were believed to have been exposed. Corneal oedema also seemed to be a reliable indicator of test outcome (see Annex 1 for methods and further details).

As the virus was still widespread and very few severe infections had occurred (five with neurological signs and 120 with corneal oedema, from which they recovered), birds with mild clinical signs were no longer removed and isolated. Isolated birds were kept together, but were allowed outside to improve their welfare, and some pens were left empty to create further spatial separation between groups. Corneal oedema was used as an indication of a group’s infection status and groups without clinical signs for 14 days were then tested, and if they tested negative the state vet permitted them to be released. All birds tested negative after sampling on 30 January 2023 and quarantine was lifted after ten weeks.

By the end of the outbreak, 153 penguins had been reported as clinically effected. These included 142 (43% of exposed) with cloudy eyes and nine that died after showed severe neurological signs (3% of exposed). One died naturally and eight were euthanised. One penguin recovered from mild head twitches and one developed severe respiratory distress and was also euthanised (Roberts *et al.*, 2023).

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4. Discussion

African Penguins, Cape Gannets, Cape Cormorants and Bank Cormorants are classified as Endangered on the IUCN Red List. All were impacted by the South African HPAI (H5N8) epidemic in the austral summer and autumn of 2018 (Khomenko *et al.*, 2018) and by the H5N1 virus in 2021 and 2022. There was considerable concern that the disease could hasten their decline, and attempts to mitigate its impact were made, but the management of wildlife diseases is usually challenging and options are particularly few for HPAI. A better understanding of the epidemiology of the disease in wild birds and systematic assessments of any available control measures are needed.

4.1. HPAI Epidemiology in South African seabirds

Mainly Common Terns were affected by HPAI in April and May 1961, when at least 1 300 died along a similar stretch of coastline. This led to the first isolation of AI virus from wild birds (Alexander, 2000; Rowan, 1962). The higher mortality of Swift Terns, compared with Common Terns, in 2018 could therefore be considered surprising but Swift Terns are the only resident species of tern that breed in such large numbers on the west and south coast of southern Africa (Hockey *et al.*, 2005; Ryan, 2017). Carcasses (e.g. of Common Terns) may have been misidentified by inexperienced observers, who had heard that the disease had initially been detected in Swift Terns, but the 60% chick mortality in two of the four main Swift Tern colonies (Department of Forestry, Fisheries & the Environment (DFFE) unpublished census data, 2022) definitely seems in excess of the normal 20% first year mortality, estimated by Payo-Payo *et al.* (2018). Although most Swift Terns were found dead, without clinical signs having been observed, and relatively few tests were done, the high proportion of positive tests in terns and mortality rate above normal suggests it is safe to assume the majority died from HPAI.

Census data from DFFE show that the two worst-affected Swift Tern colonies were in fact much larger in 2018 (at least twice and four times as large, respectively) than in previous years. In contrast, sites that usually have the largest colonies had 60-80% of the previous year's number of breeding pairs, while no breeding was recorded at other, usually smaller, colonies. It is therefore possible that high colony density contributed to the high mortality rates at the two affected sites. No breeding was recorded at these sites in 2019. The census data also indicate a decrease in the total number of Swift Tern breeding pairs since 2018; in 2019 it was approximately half that of 2018. However, by 2021, the population appears to have recovered somewhat and was similar to relatively low numbers recorded in 2012/2013. It is possible that the 2019 census underestimated numbers but also that the population only appeared relatively healthy because younger birds bred earlier than usual. Swift Terns are believed to breed after they are three years old (Hockey *et al.*, 2005), but breeding age can vary in long-lived birds (Becker and Bradley, 2007) and Swift Terns can live well into their 20s (Hockey *et al.*, 2005). Loss of older and more experienced breeders and premature breeding of youngsters will likely retard population recovery. Early breeding has been associated with both unsuccessful attempts and with higher mortality in the breeding birds (Becker and Bradley, 2007).

The 2021 HPAI (H5N1) virus did not affect Swift Terns as severely as the 2018 virus, and fewer Common Tern cases were reported. It is possible that the virus was less pathogenic to terns or that there was residual immunity from the 2018 outbreak. However, the 2021 outbreak occurred in a different season from 2018, which may also have played a role. The first seabird cases were detected in the Western Cape in autumn of 2021 and most tern cases were counted in spring/summer, at the peak of the seabird outbreak. The 2021 outbreak therefore occurred outside the Swift Tern peak breeding season and as Common terns were arriving from Europe (Hockey *et al.*, 2005). The Swift Terns were not congregating at high densities in that season (and lower population numbers would also affect density) and it is possible there were still smaller numbers of Common Terns present.

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The African Penguin mortality rate did not accelerate in 2018, even after they congregated for breeding, and no chicks tested positive. There was also no obvious subsequent change in the trend of the population or colony breeding pairs. Nest densities are highest in Simon's Town and at Stony Point and Dyer Island, where most cases were recorded, and this could have played a role (Alexandrou *et al.*, 2022; Hill *et al.*, 2019; Maxted *et al.*, 2012), but the final number of suspected cases was relatively low, given a population size similar to Swift Terns (Ryan, 2017). Additionally, given the high proportion of negative tests, the suspected cases could be excessive. As a charismatic and easily-identified species, there may have been more energetic reporting of sick and dead penguins. However, Namibia's outbreak, almost a year later, is believed to have killed at least 450 penguins between January and April 2019, mostly on Halifax Island, and they seem to have been the only species affected (Cannon, 2019; Umberto *et al.*, 2020), so African Penguins were not necessarily more resistant to HPAI (H5N8). However, it is also possible that the virus had developed new capabilities. Peyrot *et al.* (2022) compared HA and NA gene sequences from Namibian penguin viruses with the South African seabird viruses and, although the differences were not explored, the viruses were not identical. This is reinforced by the much higher numbers of penguins affected on Dyer Island and at Simon's Town, in 2021 and 2022 respectively, with less doubt about the cause of death. However, there were potentially important distinguishing features at both sites, which may have contributed. The Cape Cormorant outbreak on Dyer Island probably provided a high viral load and high doses, which may have increased the number of penguin cases. The high nest density on Foxy Beach (part of the Simon's Town African Penguin colony) may also have played a role in the relatively high number of cases from that area. The Foxy Beach "colony" is possibly the South African colony most reminiscent of the dense, open nests on Halifax Island, Namibia, where there was a high mortality rate in 2019. The close proximity of birds may have led to rapid transmission of large doses of the virus. However, the Foxy Beach area is also the more accessible area of the reserve, and it may have been easier to detect sick and dead penguins there, while undetected cases were occurring in other areas with more vegetation cover. Whatever the true extent, the predominance of cases in adults from colonies is concerning, given that, like many seabird species, African Penguins are long-lived and only breed when relatively mature, at the age of about five years (Crawford and Whittington, 2005; Whittington *et al.*, 2005).

Investigating Cape Cormorant and Cape Gannet deaths in 2018 was challenging because there was no clear indication that the suspected increased mortality rates were due only to HPAI and no definite conclusion on cause of death has been reached. Fresh samples from gannets were scarce, and budget constraints limited samples in cormorants to birds with suspicious symptoms. The outbreak coincided with the season when young cormorants fledge and are found around Cape Town in an emaciated state (SANCCOB, unpublished data). However, such high numbers of gannet carcasses are not common in southern Africa, especially away from the colonies. Avian cholera (caused by the bacterium *Pasteurella multocida*) has caused deaths in gannet colonies and thousands of fledglings have been killed by Cape Fur Seals (*Arctocephalus pusillus*) around a colony (Sherley *et al.*, 2019) but this level of mortality away from the colonies is unusual. Pohlmann *et al.* (2023) also found the highest viral loads in fresh Northern Gannet carcasses from colonies compared to decomposed carcasses found on beaches. It is possible that the decomposed Cape Gannet carcasses sampled were of insufficient quality to diagnose HPAI.

The DFFE census data do not indicate any obvious change in population trend in either of these species until late 2021, when the census showed less than half the Cape Cormorant numbers of the previous year. Cape Cormorant breeding counts do fluctuate, which has been linked to food availability (Kemper and Simmons, 2016) and disease outbreaks (Hockey *et al.*, 2005), but also due to a poorly defined breeding season, use of different breeding sites and challenges counting large colonies (Kemper and Simmons, 2016). However, the numbers recorded in 2021 were the lowest since 2013. This is plausible, given that the 24 000 affected by HPAI equates to approximately 30% of breeding

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adults counted in 2020 may have died in 2021 (DFFE, unpublished census data, 2022). The age of affected birds is not known but the effect is still expected to be significant.

Dyer Island accommodates the largest Cape Cormorant colony in South Africa (Crawford *et al.*, 2016), though numbers breeding on guano platforms in Namibia, where HPAI occurred in 2022 (Molini *et al.*, 2023), have been known to be larger (Hockey *et al.*, 2005). Dyer Island was the site of repeated outbreaks of avian cholera in Cape Cormorants, from 2002 to 2006, leading to the death of at least 20 000 adults (Waller and Underhill, 2007). Before HPAI, this was the most recent major disease outbreak in South African seabirds. The large numbers and high density of birds on Dyer Island may again be an important factor favouring disease spread and other contributing factors should be explored. However, it also seems that the H5N1 HPAI virus was better adapted to cormorants, given the higher numbers in other cormorant species, compared to 2018, especially in Bank Cormorants, which were mostly found away from sites of mass mortality.

Both the common gull species, Kelp and Hartlaub's, seemed more susceptible to H5N1 virus than to H5N8. They were among the first species to be noticeably affected and relatively large numbers, especially of Kelp Gulls, were counted during the peak of the outbreak in late 2021. Cases were identified relatively regularly throughout 2021 and 2022, and it is worth investigating their possible role in virus dissemination.

There was serious concern for the Great White Pelican population after the initial mass mortality, when 40 birds were found dead at a farm dam in May 2021, and more so when 24 were found on Dassen Island in June, as the breeding season was starting. The large outbreaks in Senegal and Mauritania, earlier in the year (Beyit *et al.*, 2023; Lo *et al.*, 2022) suggested pelicans may be highly susceptible. However, it is possible that it was early enough in the breeding season on Dassen Island that there were not enough chicks to fuel a larger outbreak.

Phylogenetic analysis showed that viruses detected in seabirds from September 2021 onwards could be assigned to two related sub-genotypes, designated SA4 and SA13 (Abolnik *et al.*, 2023). A nucleoprotein gene reassortment in an SA4 virus gave rise to SA13 in approximately July. Given that the seabird H5N8 virus was estimated to have emerged in October 2017 (Peyrot *et al.*, 2022), the difference in timing, related to different species' breeding seasons, could have influenced the species most affected. The first example of an SA13 virus was detected in a Cape Cormorant found on 13 September, either in Velddrif or near Cape Town, and another two Cape Cormorants from Lambert's Bay (north of Velddrif) in early October also had SA13 viruses. Interestingly, however, the only example of an SA5 virus was found in Cape Gannets sampled on the same day, from the same place. Other than the initial detections in Cape Cormorants, there was no apparent link between genotype and affected species.

Although gulls from near Cape Town had SA4 viruses in May, the first representative of a distinct SA4 seabird cluster was detected in a White-breasted Cormorant from Gansbaai, near Dyer Island, in mid-October. Penguins from Dyer Island in late October, sampled on the same day, also had SA4 viruses, that were all slightly different, but very similar to one from a Giant Petrel found on the west coast the week before. Unfortunately, the virus detected on 11 October, when the cormorants first started dying, was never sequenced, but it seems safe to assume it was of the SA4 sub-genotype. It would be very interesting to know why the SA13 genotype persisted, to become the dominant seabird genotype, and SA4 did not, though it may have caused the highest number of mortalities.

The SA13 viruses, probably responsible for the high Cape Cormorant mortalities on the west coast, seem to have given rise to the viruses responsible for the Cape Cormorant mortalities in Namibia in 2022 (Molini *et al.*, 2023) and the SA13 sub-genotype dominated seabird infections throughout 2022,

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including the Dyer Island and Simon's Town penguin cases in July and September/ October respectively.

A more systematic approach to sampling, aiming for better representativeness, could have helped both to estimate species susceptibility with more confidence, and to obtain the best possible sample set of circulating viruses to perform genetic sequencing. Approximately three times more samples were taken in 2021/22, compared to 2018 (Table 3.1), and at least twice as many seabird viruses were sequenced (Abolnik *et al.*, 2023; Peyrot *et al.*, 2022), reflecting an improvement in research funding and planning. Additionally, the project that sampled carcasses at SANCCOB had begun well before the first H5N1 virus was detected and could contribute evidence of the absence of the HPAI virus in seabirds for many months before it was responsible for detecting detected the first case in a gull. However, the nature of the research projects biased the sampling heavily towards penguins and carcasses and a more holistic or risk-based approach may better illuminate the epidemiology of the virus in seabirds.

Brain swabs and a pooled tracheal and cloacal sample had been used as diagnostic samples at the WCPVL since the HPAI outbreaks in poultry in 2017. The use of the brain sample was based on both the observed neurological clinical signs indicating central nervous system involvement, and the need for easily accessible tissue samples that would cause the least contamination of the work area. A brain sample can be taken quite cleanly and it saves opening the whole carcass, especially where a full post-mortem examination is not possible or desired and the only aim is to determine the presence or absence of the HPAI virus. Additionally, the brain is protected by the skull and may be better protected from desiccation and scavenging in older carcasses. Conclusions that can be drawn from these data are limited by the scarcity of other organ samples. However, the high proportion of birds with both positive brain samples and tracheal and (or) cloacal samples from 2018 indicates that where birds were determined to be shedding the virus via the respiratory and (or) digestive tracts, the virus was also present in the brain. This is aligned with findings by Swayne (2007), who found high virus (HPAI H5N1) replication titres in brain and heart, Van Den Brand *et al.* (2015) who found H5N1 virus most consistently at the highest concentrations in the brains of raptors and with Caliendo *et al.* (2020) who recorded a high level of virus neurotropism in Eurasian Wigeons and Barnacle Geese. There is also an indication from data presented here that tracheal and (or) cloacal samples, without organ samples, may not be reliable in detecting HPAI virus in seabirds. This should be investigated further as transporting carcasses for sampling would can the risk of virus spread (Meier *et al.* 2017), and information from other studies is lacking. Where neurological clinical signs predominate, the brain sample may be convenient and valuable, but other organ samples should be included where clinical signs are more variable. Rijks *et al.* (2022) studied the brains of four Sandwich Tern adults and two chicks, using histopathology and immunohistochemistry. They only found mild hyperaemia and did not detect the virus. The sample size was small, but clinical signs observed seem to have been dominated more by debilitation and lethargy and it was stated that "some" displayed apparent neurological signs such as opisthotonus and "flipped over backwards". Immunohistochemistry detected the virus more in the duodenum and pancreas, which may indicate a different focus of infection.

To understand the pathogenesis and epidemiology of HPAI in seabirds, it is necessary to conduct more detailed post-mortem examinations to determine sex, reproductive stage and other possible risk factors, and to sample, test and study more individual organs. However, this requires planning in terms of infrastructure, biosecurity, expert personnel and funding.

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4.2. Response to HPAI outbreaks in South African seabirds

Monitoring of the 2018 outbreak was done via a rapidly-developed, *ad hoc* system that was adapted based on the information that was initially produced. There was a lag before the monitoring began, while instructions were distributed, and baseline mortality and morbidity data were also lacking, which added to uncertainty about the actual effect of the disease. Though monitoring could be implemented more promptly in 2021, it was still only possible to estimate relative mortality rates and susceptibility in different species and to estimate the spatial distribution, and try to gauge when more intervention could become necessary, especially in colonies. This basic outbreak information should, however, be a minimum requirement. The aim was to prioritise the essential data and to try to ensure that these were recorded efficiently. Insistence on precise counts was necessary as hysterical, exaggerated descriptions were common. However, the definition of "suspected case" was kept broad, as it was not feasible to have a trained person examine every bird. The proportion of positive samples tested was used to assess the accuracy of the diagnoses, as was demonstrated for Cape Cormorants and Cape Gannets in 2028. It was also acknowledged that, the mortality counts would likely be an underestimation of the true number of birds affected, given those that would die unobserved. However, it was demonstrated in 2018 that additional epidemiological information such as age, signs of disease or cause of death and action taken can be recorded under most circumstances, so this information was requested by the reporting tool. This information is more difficult to include when there are many carcasses or sick birds to manage in a short space of time and a larger number of, likely unskilled, personnel are needed, but basic training and perhaps an assigned record-keeper could make this possible.

Reporting was possibly biased to areas inhabited or visited by people. However, data collected should aid decision-making about actions taken and be focused on vulnerable species. The intensified monitoring in penguin colonies may therefore have been sufficient, without attempting patrols on all remote stretches of coastline.

A central reporting point, which could then provide feedback, was a great advantage and the MS Excel template facilitated the rapid amalgamation of standardized information from different sources. The web-based tool, introduced in October 2021, was aimed at standardising and streamlining the process further, but was introduced too late to be fully adopted. It may also require better integration into existing data collection methods and daily tasks, to ensure it is accepted by field workers. Automatic collection of date and location helps to ensure that vital information is collected and minimises errors, and the data are transferred directly to a central point. Systematic surveillance, utilizing a network of observers (Lawson *et al.*, 2015) reporting at regular intervals from known locations, would have further improved the data. It could have provided a better picture of surveillance effort, to establish which areas were truly unaffected and which were simply neglected. Further participation by members of the public should be encouraged, public health risks allowing, and a way for them to report easily and efficiently should be explored.

Effective communication to relevant stakeholders was essential and should be included in all contingency plans. It is necessary to allow the collection of all available data about cases and affected locations and also to give feedback on the overall progression of the outbreak and on management decisions. Regular online meetings, chaired by experts in disaster response coordination, assisted greatly in 2021. Communication about the outbreak was relatively straightforward once a list of stakeholders had been assembled but was hampered before that had been achieved. The list also allowed the redirecting of questions from journalists to the correct organizations or people. When compiling media statements, providing additional information to public relations personnel (lists of frequently-asked questions) was efficient in assisting with follow-up questions from journalists. Along with a list of stakeholders should be clear definition of responsibilities, both spatially and functionally.

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There is not always a clear "owner" or responsible party when it comes to wild animals, but it was sometimes helpful to rather clarify the conservation authority for an area.

There was little experience with and preparation for the South African HPAI (H5N8) outbreaks in coastal birds in 2018. Broad guidelines for disease response had been compiled for the African Penguin Biodiversity Management Plan (Parsons and Vanstreels, 2016) but had not been implemented in colonies. This resulted in a high level of uncertainty and lack of confidence when making decisions about outbreak response and with limited management options. Veterinary Services had gained some experience dealing with the virus during the 2017 HPAI outbreaks in poultry, but under different, more controlled conditions. Carcass removal was perceived as ideal but was ultimately not considered essential in all situations, especially under the dry climatic conditions experienced at the time and with the assumption that transmission from subclinically-infected birds would continue to play a significant role in disease spread. HPAI viruses are expected to be present in the organs and muscles of dead birds and can be transmitted by ingestion of these tissues, but this may require a higher virus dose than via the respiratory route (Swayne and Beck, 2005). Scavenging species may therefore be at risk. The infected Jackal Buzzard and Sacred Ibises reported here and Spotted Eagle-Owl (*Bubo africanus)* and Pied Crows (*Corvus* albus) diagnosed in 2018 (Khomenko *et al.*, 2018) may represent larger numbers of affected scavenging birds that would have been more difficult to detect than colonial species. Scavengers may also cause the spread of the virus to other species, perhaps creating aerosols or droplets while feeding, or playing a role in mechanical transmission, possibly by contaminating water sources. However, further research is required to determine whether an undisturbed infected carcass could be a source of virus for non-scavenging species and, if so, under what environmental conditions. Rijks *et al.* (2022) tried to evaluate the effect of carcass removal in Sandwich Tern colonies affected by HPAI (H5N1) in the Netherlands in 2022. The results were not clear as carcass removal effort was very variable, but it appeared that survival was slightly better in colonies with increased carcass removal effort. Furness *et al.* (2023) could not detect HPAIV in water from under decomposing gannet carcasses four months after the outbreak on Foula but concluded that removal of fresh carcasses may still be of benefit. However, a risk analysis by Adam *et al.* (2023) concluded that clearing carcasses was unlikely to reduce virus transmission, considering other, more likely transmission routes.

It was not possible to provide blanket guidelines for carcass disposal in South Africa, as the conditions differ between locations, but better planning could have ensured that the best option (also considering no carcass removal) was chosen. Suitable ground for burial was often lacking, especially on the island seabird colonies. Space and plant material were also unavailable for composting, and transporting carcasses to approved landfill sites was not encouraged, to prevent the spread of the virus. Additionally, incineration facilities were few and costly but were used for smaller volumes of carcasses. Staff to collect carcasses were not always available, but concerns were also expressed that regular carcass collection may also cause harmful disruption to nesting seabirds within the colonies. The scale of the Cape Cormorant mortalities in 2021 was felt to necessitate carcass removal and this required considerable organisation and effort, for an extended period. Planning should allow for staff to be pulled away from other duties to attend to disasters of this sort, and for the other necessary resources to be provided, but also for personnel to be mentally prepared for an extended operation.

The decision to allow the mainland seabird colonies to remain open to tourists was based on the expectation that, with the already widespread distribution of the disease and the high mobility of the birds, visitors were unlikely to contribute significantly to virus spread. Raised walkways limit contact of visitors with potentially infectious material and preventing visitors would have cut off an important revenue stream necessary to maintain and protect the colonies. However, if the mortality rates had been higher, or anything like that on Dyer Island in 2021, and dead or suffering birds had been easily visible or virus loads believed to be higher, the colonies would likely have been closed to visitors. Any increase in the risk of HPAI as a zoonosis will possibly necessitate a more cautious approach.

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The 2018 blanket ban on research was possibly too drastic in hindsight. It disrupted some valuable long-term projects, but minimizing direct human contact with the birds seemed reasonable at the time. The spread of the virus between birds was considered a more important mode of transmission than human-assisted transmission, but human disturbance and handling were considered a source of stress that could result in immunosuppression.

Rehabilitation centres had to be managed differently from poultry farms, especially during times when frequent cases are reported in the wild, though the inclination initially may be to resort to principles applied to farms, such as quarantine and culling. The cost of pre-release testing was prohibitive, and the risk posed by releasing a healthy carrier was considered negligible, while there were assumed to be high levels of virus in the environment. The threat posed by virus introduction to the centres was also a sufficient incentive for the centres to take all possible precautions without being under official quarantine. They appear to have been successful in 2018, as no birds developed signs of avian influenza while in the facilities. However, determining which patients could be safely admitted to a rehabilitation centre during the outbreak was challenging without sensitive rapid point-of-care tests for AIV, which are currently not allowed at all in South Africa. This may have prevented the SANCCOB outbreak in late 2022, though it is possible the virus was introduced by a sub-clinically infected bird. In that case, testing protocols may need to apply to all birds admitted, at least during periods of higher risk, to prevent outbreaks in a rehabilitation centre. Sensitive pen-side antigen tests (lateral flow devices) would be extremely useful for screening, though possibly expensive. The sensitivity of the products available currently is also a concern, however, as a screening test should have a low probability of testing negative, if the bird is infected (World Organisation for Animal Health (WOAH), 2021). Han *et al.* (2020) performed a meta-analysis of available studies on “lateral-flow assays” and “flu virus” and calculated a pooled sensitivity of 84%. If use of the devices was approved and affordable, rehabilitation centres would need to understand that the tests may still miss approximately 16% of or one in six infected cases. The sensitivity of clinical screening should be compared when possible to evaluate the true benefits of the pen-side tests.

Contingency plans for future outbreaks should include ongoing training of relevant personnel in good biosecurity, data collection, identification and management of affected birds and other possible appropriate interventions. The correct use of personal protective equipment may also become vital, if the zoonotic risk of HPAI increases. Basic biosecurity should be maintained, even between outbreaks, as they may go unrecognised in the early stages and gathering baseline mortality data outside of outbreak periods should be a priority. This will ensure that increased mortality rates are detected early and that the correct data are collected from the very beginning of any outbreak, to allow a better understanding of the epidemiology of the disease. Stocks of sampling equipment and personal protective equipment should be maintained and carcass management plans should be in place. Samples must be collected and packaged correctly by trained staff and transported to an approved laboratory with sufficient testing capacity. Fresh samples and/ or the use of a protein-rich viral transport medium containing antibiotics for swabs are vital to provide virus for genetic sequencing to help elucidate virus transmission pathways.

A formal surveillance and testing program, with required resource estimates, should be developed as part of contingency plans. Sufficient, representative samples should be obtained from both clinically-affected birds, and apparently healthy individuals of both affected and possible subclinical carrier species. However, the associated risk of mechanical disease spread, and colony disturbance must also be considered. Centrally, there needs to be clear communication about different organizational and personnel roles. Ideally, there should be one coordinator per province dedicated to managing the outbreak and receiving regular progress reports. Resource requirements should be an explicit part of contingency planning, and there needs to be clarity on how funding will be accessed and distributed.

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Additionally, all possible management strategies should be considered and debated. One possible option is the use of vaccination, aimed at preventing deaths at a minimum but with the potential also to reduce clinical signs, infections and virus shedding and transmission. There are considerable challenges to overcome to ensure that this would be an effective and feasible approach, but vaccination should be properly investigated, given the lack of other effective interventions. With the expansion of HPAI infections to wild mammals and sporadic human cases, the need for improved and expanded surveillance and action is becoming even more apparent.

5. Conclusions

Endangered species worldwide are now threatened by HPAI and experience of outbreak monitoring and management of sick and dead birds is accumulating fast. However, knowledge about the virus's epidemiology and disease management tools are still lacking and this prevents the assembly of truly effective contingency plans. Baseline precautions should include passive surveillance by a network of trained and sensitised conservation staff, volunteers and members of the public, to ensure early detection of morbidity and mortality. Early and systematic virological investigation should then improve knowledge of transmission pathways and the role of subclinical carrier species. The importance of carcass removal is still undetermined, but can involve significant investment. Study of virus survival in and transmission from carcasses requires research and could help determine if resources are better invested away from carcass collection and disposal. A review of current data on virus persistence under coastal environmental conditions and identification and filling of data gaps is also needed. This information will contribute to decision-making about future mitigation options, including vaccination.

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Chapter 4

Survey of high pathogenicity avian influenza (H5N8) in African Penguins

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1. Introduction

South Africa was among the first countries to experience high pathogenicity avian influenza (HPAI)-induced mass mortalities in seabirds, both in 1961 and again in 2018, though clade 2.3.4.4 HPAI viruses are now recognised as a conservation threat worldwide (Banyard *et al.*, 2022; Falchieri *et al.*, 2022). The first detection of HPAI virus in penguins was also made here in January 2018 and although, by the end of the outbreak, relatively few deaths in African Penguins could conclusively be attributed to HPAI (H5N8), the species was found to be undoubtedly susceptible to the disease (Roberts *et al.*, 2023a). This was deeply concerning, given the species' Endangered status on the IUCN Red List (Birdlife International, 2018).

African Penguins' susceptibility to HPAI was further confirmed by high mortality on Halifax Island, Namibia, in 2019, assumed due to the H5N8 virus (Umberto *et al.*, 2020), and further outbreaks in South Africa in 2021, due to an H5N1 virus. Other penguin species were also found to be susceptible to H5N1 virus after cases in Chilean Humboldt Penguins (*Spheniscus humboldti*) in March 2023 and at least 10% mortality in that population (Leguia *et al.*, 2023). In addition, Chile reported HPAI in more than 460 Magellanic Penguins (*S. magellanicus*) in July 2023 (Breed *et al.*, 2023). Previous studies had demonstrated avian influenza virus exposure and low pathogenicity avian influenza (LPAI) infection in Antarctic and sub-Antarctic penguin species (Abad *et al.*, 2013; Barriga *et al.*, 2016; de Seixas *et al.*, 2022; Hurt *et al.*, 2016, 2014) but few signs of disease associated with infection. No evidence of infection or exposure had been found in penguins species further north, in southern Africa, South America or the Galápagos Islands (Karesh *et al.*, 1999; Parsons *et al.*, 2016; Smith *et al.*, 2008; Travis *et al.*, 2006; Uhart *et al.*, 2020). Additionally, there had been few detections of LPAIV in seabirds in southern Africa and little research conducted, though a basic understanding of LPAI epidemiology in the region had been built up by a handful of researchers (e.g., Abolnik, 2010; Caron *et al.*, 2011; Cumming *et al.*, 2008; Pfitzer *et al.*, 2000).

Formulating a response to the 2018 HPAI (H5N8) outbreak in South African seabirds was extremely difficult with few examples of similar outbreaks and no knowledge of AIV epidemiology in southern African seabirds, let alone penguins. Mechanisms of virus introduction and spread, and true prevalence and morbidity and mortality rates were unknown. This made it difficult to decide what actions and areas to prioritise, and where to allocate limited funding, as there could be no estimation of risk (Roberts *et al.*, 2023a).

This study aimed to estimate the prevalence of avian influenza virus and avian influenza seroprevalence in adult African Penguins across their breeding range via a cross-sectional survey. Penguins of various ages, admitted to a seabird rehabilitation centre, were also sampled to augment the numbers sampled in colonies and extend antigen testing to immature birds. The initial aim was to gauge how many penguins had been infected with the clade 2.3.4.4.b HPAI (H5N8) virus, based on presence of antibodies, relative to observed morbidity and mortality, and to determine if the virus continued to circulate after mortalities were no longer detected. However, as the HPAI (H5N1) incursion was detected soon after sampling began, the study eventually focussed largely on that virus. The association between infection status and clinical signs was explored to assess subclinical virus circulation and sensitivity of clinical diagnosis. Age, sex, body mass and origin were investigated as potential risk factors for infection with clade 2.3.4.4b HPAI H5 viruses.

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2. Materials and Methods

2.1. Study design and sample size determination

A cross-sectional study was designed to estimate the prevalence of AIV in African Penguins.

Sample size was calculated independently for the Namibian and South African populations, given the limited contact between them. The calculated sample size was then distributed proportionally between the major colonies. The initial intention was to sample the Western and Eastern Cape populations as two populations, but the conservation authorities objected on the grounds that the sample numbers required would have caused excessive disturbance in the colonies. To increase sample size, penguins admitted to the SANCCOB seabird rehabilitation centre in Table View, Cape Town were included.

For sample size (n) calculation, the standard formula for estimating prevalence from cross-sectional studies was used (Equation 1, Dohoo *et al.*, 2003), with the expected prevalence set at 50% (i.e. prevalence unknown) ±5%, with finite population correction (Equation 2).

$$n = \frac{Z^2 P(1-P)}{d^2}$$ .....equation 1

Where P= prevalence, d = precision; Z = 1.96

$$n_{adj} = \frac{(N \times n)}{(N+n)}$$ .....equation 2

Where N= population size

For sampling at colonies, an additional ten percent was added to the sample size, to allow for birds from which blood sampling was unsuccessful.

Total sample size and samples per major colony (in proportion to colony size) were calculated originally based on the 2017 census in Namibia and the 2019 census in South Africa (Figure 4.1, Table 4.1) However, during the study, the 2021 African Penguin census in South Africa revealed a further population decline, mostly due to a decrease in the numbers on St Croix Island in Algoa Bay. The overall calculated sample size did not change, but the original sample size for Algoa Bay had to be decreased and confined to Bird Island, and sample sizes at colonies in the Western Cape therefore needed to be increased. The sample sizes calculated based on the 2021 census are shown as “calculated n” in Table 4.2. In addition, the 2021 HPAI virus was detected between the completion of sampling (of the originally proposed 58 birds) on Dassen Island in January and the start of sampling in other Western Cape colonies. This may have caused the eventual estimated prevalence of AI in South Africa to be biased. To mitigate this, it was proposed that all the extra samples required in the Western Cape (because of the decrease in Algoa Bay) would be taken on Dassen Island. This created a second sample of 30 birds on Dassen Island, which, assuming random sampling, was sufficient to detect a difference in prevalence, before and after the H5N1 detection, of 18%, with 95% confidence and 80% power. It was also sufficient to detect the disease with 95% confidence, if it were present at a prevalence of 10% or more. Sample sizes were checked using EpiTools online epidemiological calculators for comparing proportions and establishing disease freedom (Sergeant, 2018). This change was approved by the managing authorities in amended permits.

Final sample sizes are shown in Table 4.7.

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Table 4.1: Sampling Regions and Sample Size

Epi. zone	Breeding pairs	Estimated population#	Sample size
Namibia*	5 400	17 500	377
South Africa**	13 300	42 500	382
South Africa (SANCCOB)	-	-	382
Total	**18 700**	**60 000**	**1141**

*2017 census, ** 2019 census

#calculated by multiplying estimated breeding pairs in 2017 by 3.2 (Crawford and Boonstra, 1994)

Table 4.4.2. African Penguins sampled for avian influenza testing: sample sizes and distribution of samples across locations

Area	Colony Site	Proportion of N*	Calculated n	Max. permitted	Handled	PCR	Serology
Namibia	Halifax Island	0.27	102	113	102	102	(102)
	Ichaboe Island	0.06	24	27	0	0	0
	Mercury Island	0.49	185	203	0	0	0
	Possession Island	0.17	66	72	51	51	(49)
Total Namibia (March 2021 & April 2022)			*377*		*153*	*153*	*151***
Eastern Cape	Bird (& St Croix) Island	0.35	132	143	141	132	137
Western Cape	Dassen Island	0.19	73	100	100 (70+30)	88 (58+30)	93 (64+29)
	Dyer Island	0.10	38	27	27	27	27
	Robben Island	0.10	38	43	41	40	40
	Simon's Town	0.10	38	36	36	34	34
	Stony Point	0.16	61	66	65	61	61
Total SA Colonies (Jan 2021-May 2022)			*382*	*415*	*410*	*382*	*391*
SA: SANCCOB	*Multiple sites*	-	*382*	-	*382*	*382*	-
	Total		**1141**		**945**	**917**	**391**

*Namibia: calculated on 2017 census, SA re-calculated on 2021 census

** Serum was sampled but has not yet been tested

2.2. Sampling

2.2.1. Fieldwork

Bird selection and distribution of samples:
Only birds in adult plumage were sampled (to target birds potentially exposed to HPAI H5N8 in 2018) and sampling was done during peak breeding season to maximise access to the adult population. Penguins were caught individually, by hand, when on/ in a nest and were immediately returned after sampling. Capture was only attempted if birds were cornered or aggressively defending a nest and were therefore unlikely to run away during capture or when they were returned after sampling. Birds were not captured if birds in neighbouring nests would likely be scared away. Sampling on Halifax Island consisted of carefully removing birds from the periphery of the colonies, to avoid excessive disturbance, and they were not necessarily on a nest.

Birds requiring rehabilitation (maximum three) were not sampled and therefore sampled birds were generally healthy.

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No bird sitting on eggs or small chicks were sampled: only birds with an empty nest or chicks that were sitting next to the parents (from approximately 10 days old; Hockey *et al.*, 2005) were selected.

Moulting birds were avoided and those that appeared to be immediately pre- or post-moult (with thickened flippers or small white feathers still present around the eyes or with prominent breast bones due to fat reserves lost during moult and not yet replaced).

If a pair was present in/ on a nest, only one was sampled. From the beginning of August 2021 (Dyer Island), on expert advice, pairs without chicks were not sampled at all, lest a breeding attempt was disrupted.

Samples were spread through the colony as evenly as possible. Nests closer than approximately 3 metres to one already sampled on that trip were excluded. Sampled nest boxes were marked with stones, that were later removed or markings were made in the sand in front of the boxes or nests.
In Namibia, a livestock marker spray was used to mark sampled birds. If available, a stick/ wand microchip reader was used to check for a microchip/ passive integrated transponder (PIT) before the bird was captured. A handheld reader was also used to scan the bird after capturing but before sampling, to further exclude birds sampled on previous trips and to prevent insertion of a second microchip. Numbers of pre-existing microchip were recorded and compared to those of previously-sampled birds, if applicable.

The regulatory authority for Bird Island (SANParks) requested that colony disturbance be recorded on the island and records were kept of any penguin seen to move away in response to the researcher's capture or replacement of a sampled penguin.

Sampling process:
The sampling team usually comprised:

- 1 person to catch and hold the penguin for measurements and sampling, who had either handled penguins for a previous research project, or who worked in the colonies, or at SANCCOB
- 1 veterinarian to take samples
- 1 person to assist the veterinarian by managing sampling tubes, labels, microchips etc. and record-keeping

In Namibia, when possible, two teams were deployed simultaneously in different parts of the colony.

Ten minutes was the prescribed maximum handling time, recorded as the time taken from removing the bird from its nest to returning it.

Body measurements included bill length and depth, head length, as described by Campbell *et al.* (2016), and body mass. These allowed an estimation of the sex of the bird (Campbell *et al.*, 2016) and of body condition (Campbell, 2016; Lubbe *et al.*, 2014).
Tracheal/ oropharyngeal and cloacal swab samples were collected from all birds by swabbing the proximal trachea or tracheal opening, choanae and oropharynx and the vent with separate sterile cotton, plastic-handled swabs.

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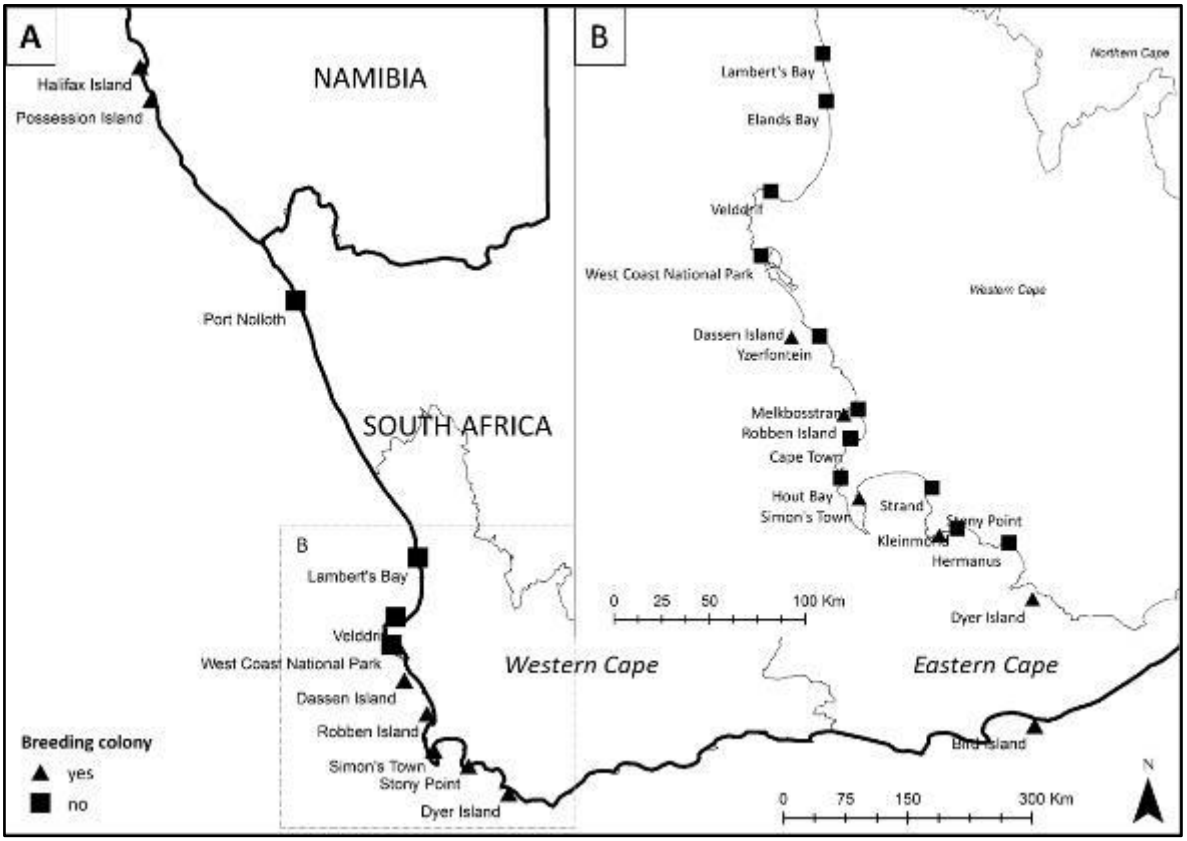


Figure 4.1: Origins of African Penguins tested for avian influenza in 2021 and 2022.
Block A includes all breeding colonies sampled in Namibia and the Western and Eastern Cape provinces of South Africa and Block B shows all origins of sampled penguins in the Western Cape.

Blood (maximum 20 ml) was collected from the jugular vein. It generally is accepted as safe to collect one percent of the body weight of a bird in blood volume (Voss *et al.*, 2010). Parsons *et al.* (2016) calculated an average body mass of 2.86 ± 0.37 kg for healthy wild adult African Penguins, so sampling 20ml of blood from the average penguin was considered safe. Additionally, Mazzaro *et al.* (2013) sampled 10ml of blood from captive African Penguins once a week for 5 weeks and found no adverse effects or significant changes to baseline haematological values. However, smaller volumes may be safer in small individuals and those in poor body condition (Voss *et al.*, 2010). The jugular vein was the preferred sampling site, over the median metatarsal or brachial veins, due to the relatively large volume of blood required.

A jugular blood sampling training session was held at SANCCOB before any fieldwork was performed. To collect a blood sample, the penguin was held between the seated handler's legs with the flippers confined next to the bird's body. The bird's head was held in the right hand of the handler and the neck stretched upwards, to straighten the sigmoid shape of the cervical spine, and extended over the right leg. The bleeder occluded the left jugular vein with the left thumb and inserted a 21 G, 1 1/2 inch hypodermic needle, attached to a 20ml syringe, into the raised jugular vein. The right vein was only attempted if bleeding was unsuccessful after two needle insertions into the vein on the left side. The right vein is larger but runs close to the oesophagus and is awkward to access for a right-handled sampler.

After blood was collected into the syringe, the bleeder and then handler placed pressure onto the venepuncture site and returned the head and neck to a normal upright position. Pressure was held on the venepuncture site for 2 minutes, during which the blood was transferred out of the syringe.

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A microchip, if not already present, was inserted subcutaneously between the left leg and the tail and six feathers (three black and three white) were plucked and placed in a small zip lock bag. The bird was then weighed on a hanging scale, suspended by a looped strap under the flippers, and returned to its nest.

Time allowing, any observed ectoparasites were collected and placed in 70% ethanol, in an Eppendorf tube. Faeces were opportunistically collected in 4% formalin, in an Eppendorf tube, for endoparasite examination and microbiome analysis.

A list of samples, equipment and storage methods is included in Annex 2.1.

2.2.2. Sampling at SANCCOB

Sampled African Penguins included 382 birds admitted to SANCCOB for rehabilitation, for various reasons, between 17 August 2021 and 26 October 2022.
Head and bill dimensions and body mass were measured and blood smears and microhaematocrit tubes were collected as part of standard admission procedures, except in some cases where HPAI was suspected and minimal handling was done to avoid contamination. Microchips were inserted after rehabilitation, before release.
In addition, blood from the microhaematocrit tube was collected on FTA paper and oropharyngeal/ tracheal and cloacal swab samples were taken to assist with preventing and controlling avian influenza in the rehabilitation centre while the virus was circulating in the wild. Swab samples were usually taken on admission, but sometimes later, within 3 days of admission. They were submitted to the laboratory, on average, 4 days after a bird was admitted, with 86% submitted within a week.

Birds eligible for sampling at SANCCOB included all ages, from when chicks were large enough to be swabbed (judged by the veterinarian), and birds judged to be strong enough to cope with the additional stress or that were suspected of being infected with HPAIV. Where large batches were admitted, approximately half were sampled and where a particularly large group arrived (112), sample size was calculated to detect at least a 5% prevalence with 95% confidence. Systematic sampling was employed as it was not practical to sample randomly.

Patient history data, including admission date, origin location, reason for admission, age, body measurements, results of admission clinical examination, final diagnosis, were extracted in bulk from the SANCCOB electronic database at the end of the study. All penguins admitted from 17 August 2021 to 26 October 2022 were included.

2.2.3. Sample handling

Orotracheal and cloacal swabs were immediately placed together in 2 ml cryovials, with 1 ml viral transport medium (VTM) containing brain-heart infusion (BHI) broth (pH 7.2) (Oxoid Ltd), 10% (v/v) glycerol and the following antimicrobials per litre: 100 mg doxycycline (Mylan), 100 mg enrofloxacin (Cipla), 1000 mg penicillin-streptomycin (Sigma-Aldrich, Merck), and 5 mg Amphotericin B (Bristol-Myers Squibb) (Pieterse *et al.*, 2022). Swab handles were removed using side cutters, which were wiped with methylated spirits on cotton wool. Swabs in VTM were kept cool in a cooler box with ice bricks, while in the field, and then refrigerated until transport to the laboratory, 1-12 (mean = 5) days after sampling.

Serum: The needle was removed from syringe and the blood transferred gently to 8.5ml gold-topped serum separator tubes (SST) and a 4ml red-topped clot-activator tube. Sealed serum tubes were gently inverted 180° and back 5 or 6 times to expose the blood to the silica clot activator.

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Blood was also drawn from the syringe into heparinised microhaematocrit tubes for evaluation of packed red cell volume (PCV) and total serum protein (TSP) and was also collected on strips of Whatman FTA paper. Two blood smears were made from blood collected in heparinised microhaematocrit tubes. These strips were placed in Eppendorf tubes, which were opened again later in an enclosed space, to allow drying. Blood smears were fixed in methanol, either at the end of each day or immediately after they had dried in the field, and one was stained with Diff Quik.

Blood tubes were kept in cooler bags with ice bricks in the field, and carried carefully to minimise haemolysis, then refrigerated until centrifugation at 2254 g for 15 minutes. Serum was removed using clean disposable Pasteur pipettes and transferred to 2ml cryovials. Residual red blood cells were either frozen or 100% ethanol was added (approx. 50:50) for preservation.
Serum was refrigerated, or frozen at -20°C, and kept cold when transported to the laboratory. There it was frozen at -80°C.

Each sample was labelled with the bird's study reference number (APXXX), the date (dd mmm yyyy) and the location.

2.2.4. Biosecurity

Ensuring adequate biosecurity to lower the risk of anthropogenic disease transmission between colonies and different areas of the same colony was a priority. A copy of the protocol is included in Annex 2.2.

2.3. Laboratory Testing

Serological samples and swabs were submitted to either the Western Cape Provincial Veterinary Laboratory (WCPVL) in Stellenbosch, AssureCloud private laboratory in Oudtshoorn (226 samples from SANCCOB) or the Central Veterinary Laboratory (CVL), Windhoek, Namibia (Namibian samples).

2.3.1. rRT-PCR

Molecular methods used at WCPVL were used as described in Roberts *et al.* (2023): swab samples were first screened with influenza A virus real-time reverse transcription polymerase chain reaction (rRT-PCR) VetMAX™Gold AIV detection kit (Thermo Fisher: Life Technologies, Cat. no. 4485261) according to the manufacturer's protocol. The kit targets the matrix protein 1, matrix protein 2 and nucleoprotein genes of influenza A viruses. Cycle threshold (Ct) values < 36 were considered positive. IAV-positive samples were then tested with a H5- and H7-specific rRT-PCR using the VetMax-Plus One-Step RT-PCR Kit (Cat. No. 4415328), using the primers and probes described by Slomka *et al.* (2007) and Ct ≤ 35 was considered positive.

The Central Veterinary Laboratory (CVL) in Windhoek also used the VetMAX™Gold AIV detection kit.

AssureCloud: Samples were screened using the rRT-PCR RealTime ready RNA Virus Master kit (Roche Life Science, Cat. No. 05992877001), targeting the IAV matrix gene with primers and probes described by Spackman *et al.* (2002), supplied by TIB Molbiol Synthesis Laboratory, Berlin, Germany (Ref:022111063). Ct values <35 were considered positive. AIV positive sample were tested with H5-specific primers and probes (Ref:023108059), described by Slomka *et al.* (2007). Ct values <35 were considered positive.

As required by the South African national Directorate: Animal Health, all AIV-positive samples were forwarded to the Agricultural Research Council's Onderstepoort Veterinary Research Institute (ARC-OVR) and confirmatory and sub-typing tests were performed. RNA from HP H5 AIV was detected using

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real-time RT-PCR (primers and probes: Naguib *et al.* (2017)), and RNA from N1 AIV was detected using Sybr Green-based real-time RT-PCR (primers: Payungporn *et al.* (2006)).

AIV RNA was also sent to the Poultry Research Laboratory, Faculty of Veterinary Science, University of Pretoria. RNA samples were tested using an influenza A virus pan-genome RT-PCR assay described by Zhou *et al.* (2009). The PCR amplification products were submitted to Stellenbosch University Central Analytical Facilities for Ion Torrent Next Generation Sequencing (NGS).

2.3.2. Serology

Avian influenza serology:
Serological tests were performed at the Western Cape Provincial Veterinary Laboratory (WCPVL). They included an influenza A enzyme-linked immunosorbent assay (ELISA) (IDEXX Influenza A Virus Antibody Test Kit, part 99-53101, IDEXX Laboratories), performed according to the manufacturer's instructions, which detects influenza A nucleoproteins in animal (bird and mammal) serum. The diagnostic sensitivity and specificity, established at WCPVL for chicken, ostrich and duck serum were 95.24% and 93.94% respectively (M Dreyer, pers. comm). Additionally, haemagglutinin inhibition (HI) tests were performed, using standard test reagents from Deltamune (Pty) Ltd and the Southern African Development Community (SADC) protocol (Kangumba, 2011; Kangumba *et al.*, 2009), in line with the WOAH-recommended methods for non-chicken species. The HI antigens employed were:

- A/chicken/South Africa/Villiers/2017 (**H5N8**; HPAI)
- A/mute swan/England/AVP 18 001986/2017 (**H5N6**; HPAI)

Additionally, each 0.5 ml serum was treated with 0.5 µl neuraminidase from *Vibrio cholera* (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) and incubated at 37°C overnight, followed by inactivation in a 56°C water bath for 30 minutes and adsorption of any non-specific agglutinins with chicken RBCs, to avoid non-specific haemagglutination. The addition of neuraminidase and adsorption step ensure that the HI assay is suitable for all species.

ELISA tests with S/N ratios <0.5 were considered positive, as recommended by the kit manufacturer. HI titres ≥16 were considered positive (WOAH, 2021). Both the H5N6 and H5N8 titres were required to be ≥16 for the sample to be considered H5-positive, to avoid misinterpretation of results due to steric inhibition of agglutination. This is caused by the test serum containing antibodies against the same N-type as the test antigen. Samples with paired H5N6 and H5N8 titres <16 but >0 were considered suspect. Testing was repeated on samples with positive and suspect results.

Avian paramyxovirus serology:
Serum (n=387) was also tested for antibodies against avian paramyxovirus serotype 1, which includes virulent strains causing Newcastle disease (NCD). An HI test was employed, with Avipro ND Lasota (Live lyophilized lentogenic NDV strain LaSota) as the antigen. HI titres ≥16 were considered positive.

2.3.3. Packed cell volume (PCV) and total serum protein (TSP) assessment

Microhaematocrit tubes were centrifuged for 5 minutes at 12 353 g. The microhaematocrit tube was used for assessment of PCV, by calculating the percentage red blood cells out of total blood volume in the tube, and total serum protein TSP in g/dl, measured using a handheld refractometer.

2.3.4. qPCR for penguin circovirus

The serum samples collected in Namibia were screened for penguin circovirus at the Windhoek CVL, using a qPCR assay, as described by Roberts *et al.* (2023)

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2.4. Data analysis

2.4.1. Data cleaning (SANCCOB data)

- Some locations of origin were close together, so were grouped. Locations within approximately 20 km of a breeding colony were grouped with that colony and locations further away were grouped under the name of a relatively central location within approximately 20 km.
- Measurements that clearly appeared to have been recorded in the wrong units were corrected.
- Reason for admission was categorised into suitable broad categories.
- Age of chicks was re-classified, as a consistent system had not been used, and records for birds admitted as eggs were removed. Further clinical records were consulted where age could not be determined with confidence.
- Reason for admission was re-categorised into broad categories.
- Clinical examination records for each body system were searched for clinical signs associated with HPAIV detections and summarised if present.

2.4.2. Description of sampled African Penguins

Characteristics of sampled birds were summarised using descriptive statistics, and possible sources of bias investigated. Proportions observed were compared to the expected unbiased proportion (e.g. 50% for sex) using a one-sample test for binomial proportion, normal-theory method, in OpenEpi, Version 3, an open source epidemiological calculator (Dean *et al.*, 2013).

Outliers in body measurements, TSP and PCV were identified by calculating interquartile range.

The upper limits of the 95% confidence intervals for proportions were calculated using the Mid-P Exact method, assuming a large population, in OpenEpi.

Distribution of sample sites was illustrated using Esri® ArcMap™ in ArcGIS Desktop version 10.7.1, Esri Inc., Redlands, CA, 2019.

Packed cell volume (PCV) and total serum protein (TSP) were compared to the findings of Parsons *et al.* (2015). Their findings are summarised in Table 4.3.

Table 4.3: Baseline haematology and biochemistry parameters set for wild adult African Penguins by Parsons et al., 2015

Variable	mean	s.d.	Values from previous studies referenced
Haematocrit/ PCV (%)	46	5.7	43-46 (mean=45; n=5)
TSP (g/L)	59	9.61	53, 63 (mean=58; n=2)

Calculation of body condition indices (BCI): After removing outlying head and beak measurements (greater than the 3rd quartile + (1.5 x interquartile range (IQR)) and less than the 1st quartile- (1.5 x IQR)), the Lubbe body condition index (Lubbe *et al.*, 2014) was applied to chicks and fledglings with head lengths >75mm and the Campbell index (Campbell, 2016) to adults and juveniles. Moulting and perimoult birds were excluded.
Calculated BCI were categorised using bin ranges and histograms employed to assess distribution in the sampled population.

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Sex determination via beak measurements: After removing outlying beak measurements, the Farah discriminant function (Campbell *et al.*, 2016) was used to assign sex to all adults where bill length and depth had been measured.

2.4.3. Statistical analyses

The upper limits of the 95% confidence intervals for prevalence were calculated using the Mid-P Exact method, assuming a large population, in OpenEpi. “Estimated” or “approximate” are used to describe these upper limits, since sampling was not random and therefore prevalence cannot be calculated with any assurance.

Investigated risk factors for HPAI infection were location, age, sex, body condition and clinical signs. The association between AIV infection and these potential risk factors was assessed using two-by-two contingency tables (OpenEpi). Odds ratios were calculated, with 95% confidence intervals, and chi square analysis was performed if expected values were greater than five, and the mid-P exact method used if expected values were less than five.

2.5. Permits and permission required

2.5.1. Veterinary research permit under Section 20 of the South African Animal Diseases Act 35 of 1984, from the national Director: Animal Health, Department of Agriculture, Land Reform and Rural Development (DALRRD) (12/11/1/1/MG

2.5.2. Department of Environmental Affairs research permit (Res2020-54, Res2022-13-n=415

2.5.3. CapeNature research permit (CN44-59-13038, CN44-87-18843, CN44-87-19390)-n= 193 to be handled in CapeNature colonies

2.5.4. SANParks research permit (CRC/2020-2021/022--2019/V1, CRC/2021-2022/022--2019/V1 – n = 1200

2.5.5. Ethical approval from the Animal Ethics Committee of the University of Pretoria (Certificate Number V00719).

2.5.6. City of Cape Town approval 3.5.7.Robben Island Museum approval

3. Results

945 African Penguins were sampled in Namibia and South Africa, between January 2021 and October 2022.

3.1. Namibian colonies

3.1.1. Characteristics of penguins sampled in Namibia

3.1.1.1. Spatiotemporal distribution

Halifax Island was sampled in March 2021 and Possession Island in April 2022. Samples were evenly distributed across inhabited areas of Halifax and Possession Islands (Figure 4.2 and Figure 4.3). Only one colony, up on a ridge, was excluded on Halifax Island because of its history of nervousness and the possibility of causing a stampede. The marked points in the figures show where sampling took place, rather than the locations of individual nests, so often multiple birds were sampled at one site.

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Figure 4.2: African Penguin sampling sites on Halifax Island, Namibia, in March 2021, marked with stars. The excluded colony is shown by the black ring. (Satellite imagery: © Google, 2023)



Figure 4.3: African Penguin sampling sites on Possession Island, Namibia, in April 2022, marked with stars. (Satellite imagery: © Google, 2023)

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3.1.1.2. Breeding *status*

Of the 153 penguins sampled in Namibia 32% of sampled birds had chicks, 50% were alone, 10% were with another adult (pairs, at Halifax only), 1.3% (two) were building nests and breeding status was not recorded for 7% (Table 4.4).

Table 4.4: Breeding status/ nest contents of Namibian penguins at different locations, where sex could be calculated from beak measurements with confidence

Location & Sex	Chick(s)	Pair	Building a nest	Alone	Unknown	Total
Halifax Island	25	10	2	31	6	74
Female	6	2	0	5	1	14
Male	19	8	2	26	5	60
Possession Island	10	0	0	25	1	36
Female	3	0	0	8	0	11
Male	7	0	0	17	1	25
Total	**35**	**10**	**2**	**56**	**7**	**110**

3.1.1.3. Sex

Of the 110 Namibian penguins where sex could be estimated with confidence, using the Farah discriminant function (Campbell *et al.*, 2016), 25 (23%) were determined to be females ($p<0.001$ when compared to 50%). On Halifax Island, 19% were females ($p<0.001$) and on Possession Island, 30% ($P=0.02$; Table 4.5).

Table 4.5: Origin of Namibian penguins and sex estimated from beak measurements

Row Labels	Male	Female	unknown	Total
Halifax Island	60	14	28	102
Possession Island	25	11	15	51
Total	**85**	**25**	**43**	**153**

There was also a greater proportion of males when penguins were categorised as non-breeding or breeding (with chicks or found in pairs; Table 4.5). Males found with chicks comprised 74% of birds with chicks overall ($P=0.004$) and on Halifax Island 76% ($P=0.009$). There were too few birds with chicks sampled on Possession Island to detect a possibly significant difference between proportions of males and females.

The proportions of males were compared between vets performing head measurements on different islands and, by default, between handlers selecting birds (vets generally worked with the same handler on a specific island). The higher proportion of males remained for all vet-island combinations (data not shown).

3.1.1.4. Clinical assessment

71% of birds (n=108) were recorded as having no abnormalities detected and records are lacking for 14% (n=22) but they are assumed also to have had no major abnormalities. 6.5% (10) had healed wounds (2.6% = four birds with missing toes), 3% (4) were recorded as thin, 2% (3) had lesions around the eyes (probably due to tick bites) and 4% (6) had minor abnormalities with only one or two birds with each.

The mean packed cell volume (PCV) was 47 (SD=4.9). 95% had a PCV of 38% or higher. The mean TSP was 6.3 (SD=0.9). 95% had a total serum protein (TSP) of 5.4g/dl or higher.

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3.1.1.5. Body condition

Ten penguins were excluded when calculating body condition index (BCI): five recorded to be perimoult, two with full stomachs, two without a recorded body mass and one with an extremely short bill length.

The majority of the remaining sampled birds (89%, n = 127) had BCI indicating body mass between the 0.05 and 0.95 quantiles (Figure 4.4).

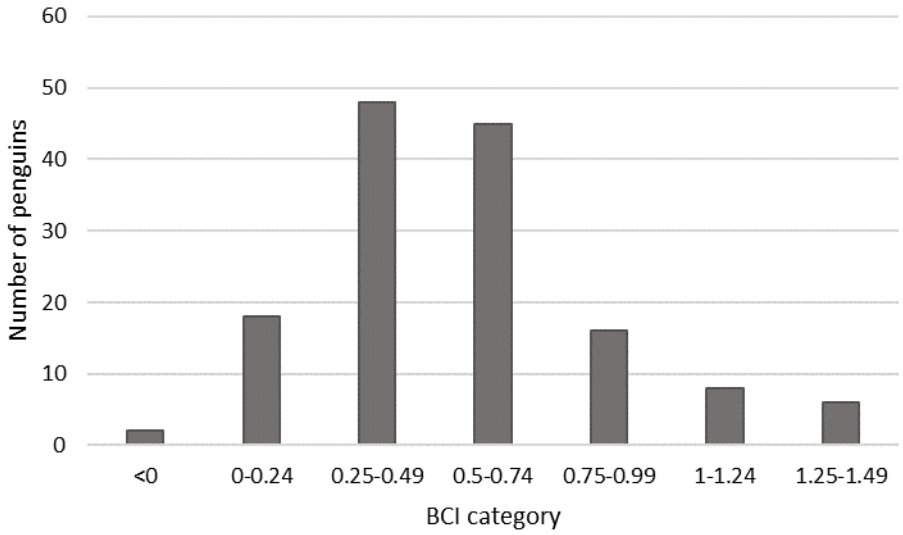


Figure 4.4: Frequency of different body condition indices (BCI) calculated for penguins sampled in colonies in Namibia (n=143)

3.1.2. Avian influenza test results

rRT-PCR: All swabs collected from penguins in Namibia tested IAV rRT-PCR negative. The overall IAV prevalence in the southern Namibian colonies was unlikely to be higher than approximately 2%, though it could have been up to approximately 6% on Possession Island (Table 4.6).

Testing of the serum for IAV antibodies is pending.

Table 4.6: African Penguin samples from Namibia and Influenza A virus (IAV) test results, where applicable

Colony	Sample date	Swabs (rRT-PCR negative)	Estimated IAV prevalence upper limit 95% CI	Serum samples (not yet tested)
Halifax Island	Mar 2021	102	0.03	102
Possession Island	Apr 2022	51	0.06	49
Total		153	0.02	151

3.1.3. Penguin circovirus results

The 151 serum samples tested negative for penguin circovirus (Roberts *et al.*, 2023).

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3.2. South African colonies

3.2.1. Characteristics of penguins sampled in South Africa

3.2.1.1. Spatiotemporal sample distribution

Robben Island and Dyer Island were sampled on single visits but other locations were visited up to three times and the visits took place between 19 days and eleven months apart (Figure 4.5).
Fifty-two percent of samples (n=214) were taken in a nine-week period from 30 July to 3 October 2021; 59% of Western Cape samples in the first six weeks, at Dyer and Robben Islands, Simon's Town and Stony Point (Betty's Bay) and 38% of the Bird Island samples (Table 4.7).

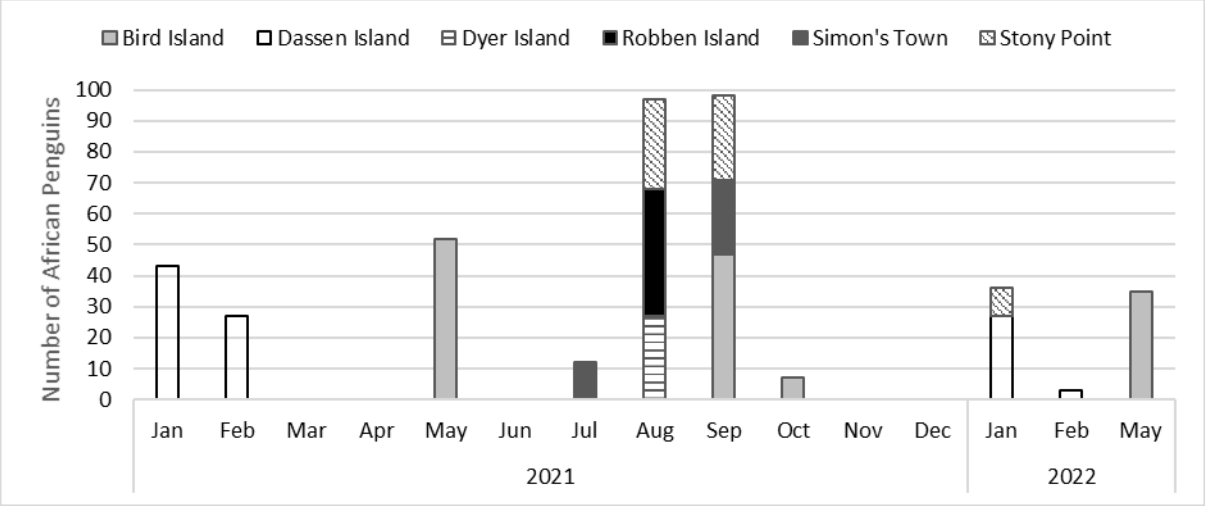


Figure 4.5: Spatial and temporal distribution of African Penguins sampled in South African colonies.

Distribution of samples at breeding sites is shown in Figure 4.6 to 11. The marked points show where sampling took place, rather than the locations of individual nests, and often multiple birds were sampled at one site. Nests were within 20m of the sampling site, though usually closer. Inaccessible areas included Foxy Beach at Boulders (deemed too public by the conservation authority), as well as densely-bushed areas at Boulders, the southeast corner of Bird Island (other species breeding and very nervous penguins), the northwest corner of Dassen Island (pelicans breeding and a zone excluded from disturbance), the south end of Dyer Island (no disturbance allowed) and a block north of the prison on Robben Island, also designated an area of no disturbance.

3.2.1.2. Breeding status and sex

51% of sampled birds came from nests with chicks, 38% were alone ("loafing"), 8% were with another adult (pairs, sampled only at the first visits to Dassen and Bird Islands), 1.5% were moulters (5/6 post-moult) and two unknown, one because it hid while commuting.

Approximately equal numbers of males and females were sampled at all colonies, except for Simon's Town where females may have made up as little as 27% (P=0.019 when compared to 50%; Table 4.8). When examining the breeding status of different sexes at Simon's Town, the lowest proportion of females was in the loafing group (2/11), which was also the largest group (data not shown).

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Figure 4.6: African Penguin sampling sites on Bird Island, 2021-2022. (Satellite imagery: © Google, 2023)



Figure 4.7: African Penguin sampling sites on Dyer Island, August 2021, shown by black stars. (Satellite imagery: © Google, 2023)

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Figure 4.8: African Penguin sampling sites at Stony Point, 2021-2022. (Satellite imagery: © Google, 2023)



Figure 4.9: African Penguin sampling sites in Simon's Town (including Boulders), 2021. (Satellite imagery: © Google, 2023)

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Figure 4.10: African Penguin sampling sites on Robben Island, August 2021. (Satellite imagery: © Google, 2023)

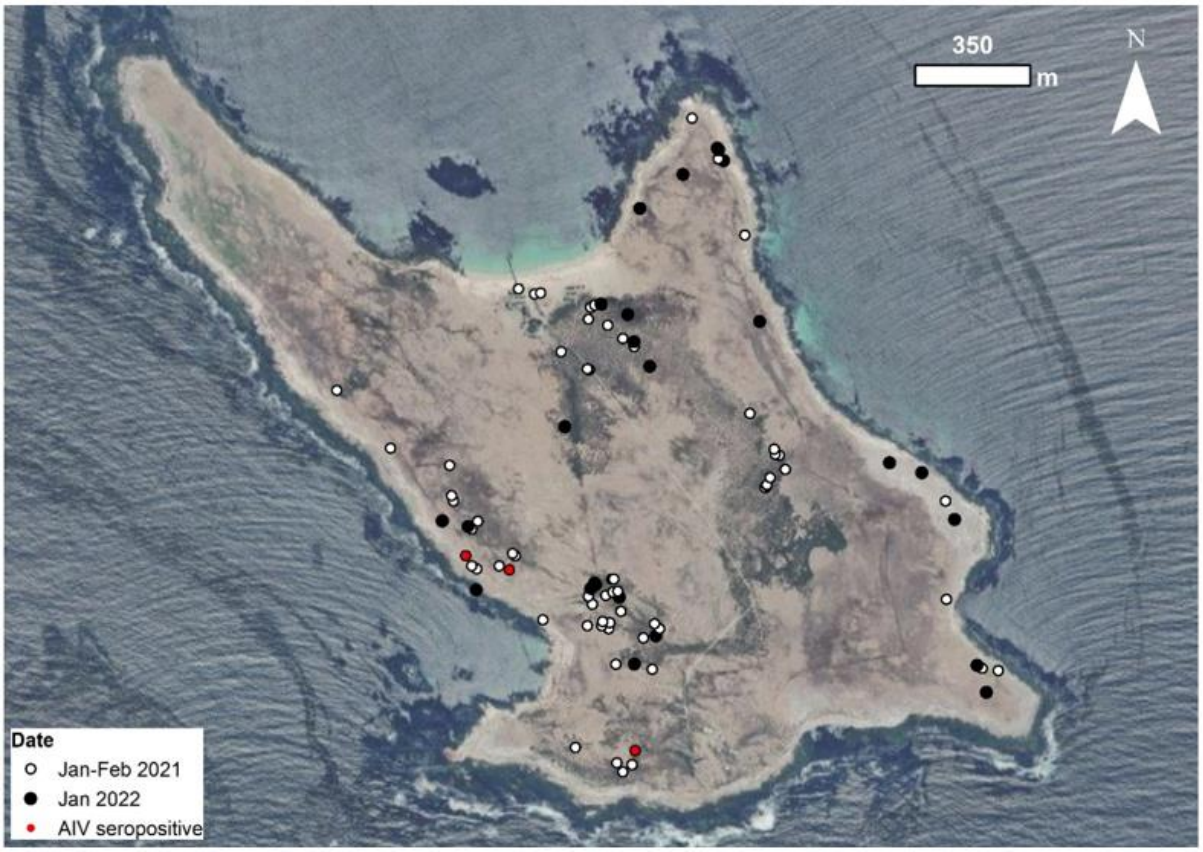


Figure 4.11: African Penguin sampling sites on Dassen Island, January- February 2021 and January 2022. (Satellite imagery: © Google, 2023)

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Table 4.7: Locations and dates of and test results for African Penguin samples from South African colonies.

		rRT-PCR		NP-ELISA		clade 2.3.4.4b HI				NDV HI
	Sampling dates	Total (neg)	Upper limit 95% CI	Total ELISA	pos	Total HI	pos (suspect)	Lower limit 95% CI	Upper limit 95% CI	Total: neg
Bird Island	May 2021	52	0.06	50	0	50	1 (1)	0.007	0.13	50
	Sep/Oct 2021	54	0.05	54	0	54	0	0.000	0.05	54
	May 2022	26	0.13	33	0	33	0	0.000	0.09	32
		132	0.02	137	0	137	1 (1)	0.003	0.05	136
Dassen Island	Jan/Feb 2021	58	0.05	64	2	61	1	0.001	0.08	61
	Jan/Feb 2022	30	0.10	29	0	29	0	0.000	0.10	28
		88	0.03	93	2	90	1	0.001	0.05	89
Dyer Island	Aug 2021	27	0.11	27	0	27	(1)	0.002	0.17	27
Robben Island	Aug 2021	40	0.07	40	0	40	(1)	0.001	0.12	40
Simon's Town	Jul 2021	12	0.22	11	0	11	0	0.000	0.24	11
	Sep 2021	22	0.13	23	0	23	0	0.000	0.12	23
		34	0.08	34	0	34	0	0.000	0.08	34
Stony Point	Aug 2021	28	0.10	28	0	28	0	0.000	0.10	28
	Sep 2021	26	0.11	25	0	25	0	0.000	0.11	25
	Jan 2022	7	0.35	8	1	8	0	0.000	0.31	8
		61	0.05	61	1	61	0	0.000	0.05	61
TOTAL		**382**	**0.008**	**392**	**3**	**389**	**2 (3)**	**0.005**	**0.028**	**387**

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Table 4.8: Sex of African Penguins sampled at different locations, estimated using the Farah discriminant function

Row Labels	Male	Female	Unknown
Bird Island	49	42	46
Dassen Island	29	36	35
Dyer Island	8	12	7
Robben Island	16	13	12
Simon's Town	19	7	10
Stony Point	26	24	15
Grand Total	**147**	**134**	**125**

3.2.1.3. Body condition

Nine penguins were excluded from BCI calculations because they were recorded to be moulting and another 23 with noticeably full stomachs were excluded, as well as five without body mass recorded (Total 37).

Eighty-five percent of the remaining sampled birds had BCI between 0 and 1 and only 4% (n=15) had a negative BCI (Figure 4.12).

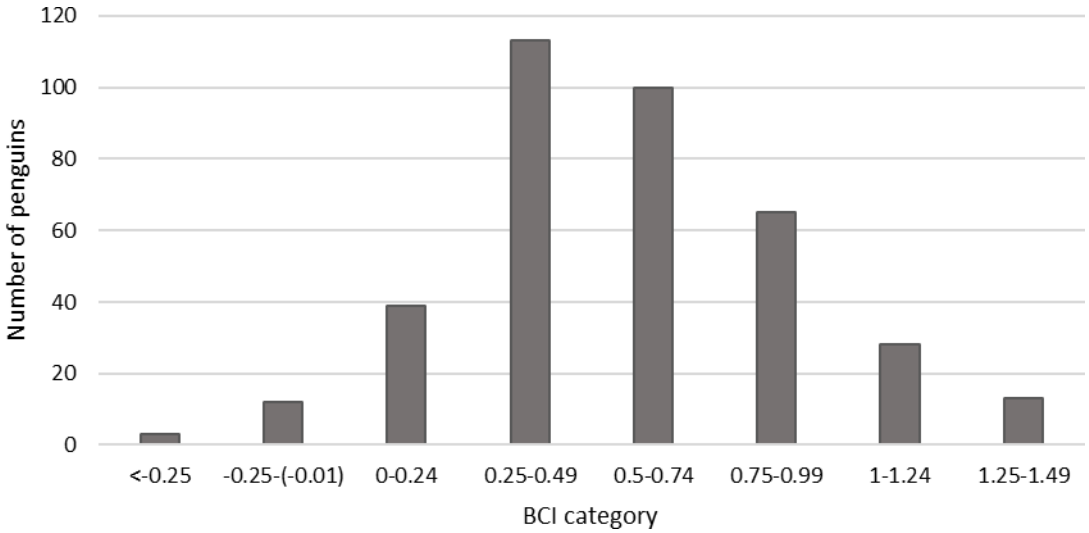


Figure 4.12: Frequency of different body condition indices (BCI) calculated for penguins sampled in colonies in South Africa (n=374)

3.2.1.4. Clinical assessment

Three hundred and two (83%) of penguins sampled in South African colonies had no abnormalities detected. 7% were perceived to be thin, 3% had minor injuries on body, mostly already healed, 2% had wounds around the eyes, half attributable to tick or flea bites, 2% had ticks and 1% fleas, one bird had one leg missing (healed) and 6% had a variety of minor abnormalities (one or two birds each).

The mean PCV was 48 (SD=6.0). 95% had a PCV of 38% or higher and at least 3/16 that were lower appear to be artefacts from poor quality blood samples.
The mean TSP was 6.7 (SD= 1.1). 95% had TSP of 5.2g/dl or higher (minimum 4.3).

Re-captures

The microchips of eleven sampled penguins (3%) were detected again, when checking nests, four and thirteen months after sampling.

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One penguin sampled on Dassen Island in January 2021 was re-sighted in January 2022, in a burrow, with two P2 chicks.

Eight penguins (6%) were re-sighted on Bird Island, four sampled in May 2021 (one sighted in September 2021 and three in May 2022) and four sampled in September 2021. Seven were with chicks and eighth seemed to be fattening for moult.

Two sampled birds were subsequently found dead: one sampled 1 September 2021 at Boulders (Willis Walk) was found dead on Foxy Beach on 11 October 2022and the other was sampled on Dyer in August 2021 and found dead on 11 December 2021. Both were found in the midst of HPAI outbreaks.
One bird with a flipper tag was sampled at Boulders on 30 July 2021. Its records showed that it was at least 17 years old and had been breeding at Stony Point in 2019 and 2020. Another bird sampled on Robben Island (August 2021) was investigated because it, unusually, had a microchip in the neck, denoting a bird possibly marked earlier in the tagging program. It was at least eight years old, tagged at SANCCOB in 2013.

3.2.2. Avian influenza test results for South African colonies

Results of IAV rRT-PCR testing of oro-tracheal and cloacal swabs and ELISA and haemagglutination inhibition testing of serum are shown in Table 4.7. Results from Dassen Island trips for 2021 and 2022 respectively were combined as they took place less than a month apart and there were no major changes in HPAI incidence anywhere during that time. The total number of birds from which serum was sampled was greater than swab samples because some blood samples were expected to be unsuitable for testing, but more than expected were eventually suitable.

3.2.3. IAV rRT-PCR

All 382 rRT-PCR tests were negative. The upper limits of the 95% confidence interval are shown, for each sampling trip and for the overall population, in Table 4.7.

The calculated maximum population prevalence for avian influenza virus in healthy adult penguins, based on these results, was 0.8% overall and 1.8% for the Western Cape colonies samples from July to September 2021. An upper limit of 2% was calculated for Bird Island and 11% for Dyer Island, though, for individual sampling trips, the lowest was 5% on Dassen Island in early 2021 (n=58) and the highest was 35% at Stony Point in January 2022 (n=7). For July-October 2021, when 52% of samples were collected at five of six sites (including Bird Island in the Eastern Cape), an upper limit of 1.4% was calculated.

3.2.4. Serology

Eight serum samples had serology results indicative of previous IAV infection: three were ELISA-positive only and five had H5 HI titres.

Serum was available from 392 birds for ELISA testing and three samples tested positive, though HI-negative. Two originated from Dassen Island in February 2021, from the same area, about 20m apart (Figure 4.12), sampled on two successive days, and the third was from Stony Point in January 2022. The ELISA-positive proportion for Dassen Island in January-February 2021 was 3% (0.5-11%) and 14% (0.7-53%) at Stony Point in January 2022. One ELISA-positive bird from Dassen Island had two P2 chicks but the other two were alone. One each were designated male, female and unknown sex and all appeared healthy. One on Dassen Island had a relatively low BCI of 0.18 but the other two were “normal” to high at 0.66 and 0.84.

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Serum volume was adequate for HI testing of 389 birds. Serum from five birds showed reactions to the H5N8 and H5N6 HI antigens, with titres >0, though all were negative on the ELISA tests. H5 HI test positive proportion was 1% (0.5-3%). Two samples had paired H5N6 and H5N8 reactions with titres above the positive cut-off of 16 (H5N8 titres of 64 and 32). One bird was sampled on Dassen Island in February 2021, in a similar area to the ELISA-positive birds but further south. The other was sampled on Bird Island in May 2021. Another three birds had suspect H5 HI titres (both antigens >0 but <16). One was from Bird Island in May 2021 (in a similar area to the HI-positive bird; Figure 4.7), and the others from Dyer and Robben Islands in August 2021. The Dyer Island sample was not treated with neuraminidase, however, as it was depleted. The July-October 2021 H5 HI test positive proportion was 0.9% (0.1-3%).

Of the five HI-positive and suspect birds, three had chicks (one of them positive) and the other two were alone. No clinical abnormalities were detected, though two HI-suspect birds (from Dyer and Robben Islands) had low BCI (0.15 and 0.02) and one of these was noticeably thin. Both also had TSP lower than the first quartile (5.4 and 5.6). Four of five, except the positive from Dassen, where sex is unknown, were female. The odds of a female testing either ELISA or HI positive were 5.5 times higher than for males (ignoring birds with unknown or incalculable sex; P=0.10; mid-P).

3.2.5. APMV-1 serology

HI tests for avian paramyxovirus serotype 1 on 387 birds were also all negative. The only reactions were three samples with titres ≤ 16, one from Dyer Island in August 2021 (not treated with neuraminidase) and two from Stony Point in September 2021.

3.3. Sampling at SANCCOB

3.3.1. Sample distribution

3.3.1.1. Clinical signs

SANCCOB admitted 847 African Penguins between 8 August 2021 and 26 October 2022. 382 (45%) of admitted penguins were sampled on or soon after admission. Sampled proportions per reason for admission were 68%, 67% and 66% of birds admitted for injuries, poor body condition and "miscellaneous reasons" respectively. 32% of those abandoned or removed from risk areas (comprising chicks or fledglings) and 81% (n=13) with suspected HPAI were sampled (Table 4.9).

Table 4.9: Reason for admission to SANCCOB and numbers of penguins admitted and sampled

Reason for admission	Admitted	Sampled	Proportion sampled
Abandoned/ removed due to risk	541	174	0.32
Injured	154	104	0.68
Underweight	92	62	0.67
Other	44	29	0.66
Suspected avian influenza	16	13	0.81
Total	847	382	0.45

3.3.1.2. Age

70%, 77% and 74% of adults, juveniles and fledglings were sampled respectively and 30% of chicks. All sampled chicks comprised P2-P4 categories, with no smaller chicks sampled and only 12% of the P2 category (before any chick down is lost). 40% of P3 and P4 chicks were sampled (Table 4.10). The lowest mass of a sampled bird was 600g, but all but one were heavier than 840g. Within age categories, samples were relatively evenly distributed between admissions for different reasons, in proportion to the number admitted for that reason (data not shown).

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Ninety-eight chicks (61% of chicks sampled) and 11 (16%) fledglings were sampled on four days when 214 chicks and 18 fledglings were removed from Stony Point (2 November 2021), Dyer Island (20 and 23 November 2021) and Simon's Town (26 October 2022) because their parents were moulting and were unable to feed them (Table 4.11). Subsets of these groups were sampled, but at higher proportions than the average for chicks admitted on other days (19%; Figure 4.14 and Figure 4.15).

Table 4.10: Ages of penguins admitted to and sampled at SANCCOB (8 August 2021 to 26 October 2022)

Age category	Total admitted	No. sampled	Proportion sampled	Proportion of samples
Adult	155	109	0.70	0.29
chick	*540*	*160*	*0.30*	*0.42*
0	9	0	0.00	0.00
1	36	0	0.00	0.00
2	122	15	0.12	0.04
3	143	47	0.33	0.12
4	223	98	0.44	0.26
3 & 4	366	145	0.40	0.40
Unknown	7	0	0.00	0.00
Fledgling	95	70	0.74	0.18
Juvenile	56	43	0.77	0.11
Unknown	1	0	0.00	0.00
Total	**847**	**382**	**0.45**	

Table 4.11: Origins of and dates that chicks and fledglings were sampled from large groups admitted after removal from colonies due mostly to moulting parents

Location	Date(s)	Admitted	Sampled	Prop. sampled
Betty's Bay	**02/11/2021**	122	46	0.37
Dyer Island	**20 & 23/11/2021**	78	40	0.51
Simon's Town	**26/10/2022**	32	23	0.72
Total		231	108	

3.3.1.3. Location

88% of admitted penguins were from four of the five largest local colonies (Dassen Island was underrepresented) and 80% of samples came from these (Figure 4.13). 33% (Betty's Bay) to 52% (Simon's Town) of birds admitted from these colonies were sampled (Table 4.12). Higher proportions of birds from Simon's Town were sampled for all admission reasons, except for abandoned chicks (data not shown).

3.3.1.4. Sex (adults only)

Fewer males were admitted than females (using the Farah discriminant function to determine sex) and sampling was in proportion to numbers admitted (Table 4.13).

3.3.1.5. Body condition

42% of admitted penguins had a BCI between 0 and 1 and 55% had a BCI less than 0 (Figure 4.16). Proportionally more birds with BCI less than -0.5 were sampled (Table 4.14).

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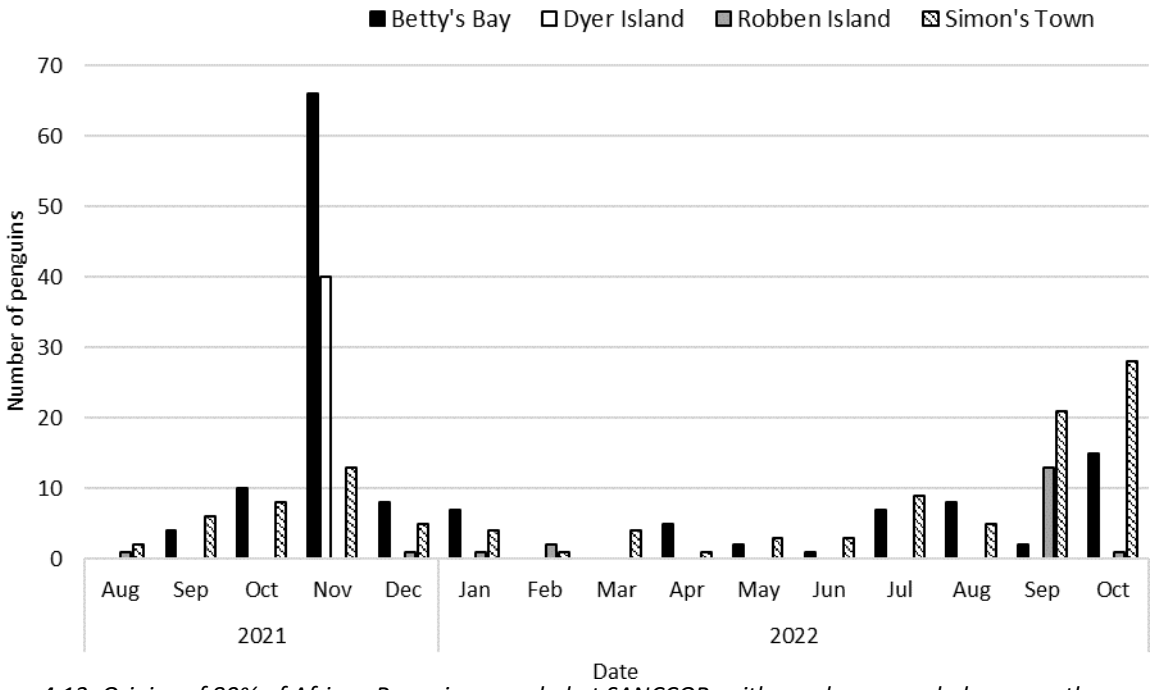


Figure 4.13: Origins of 80% of African Penguins sampled at SANCCOB, with numbers sampled per month

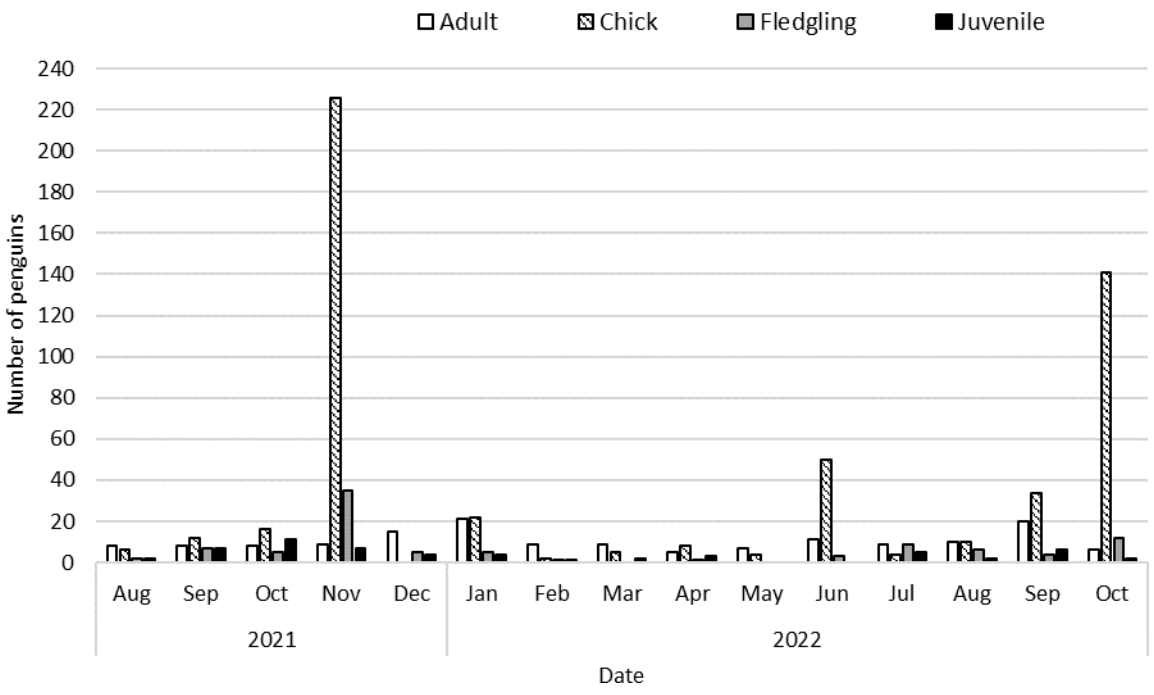


Figure 4.14: Age categories of African Penguins admitted per month

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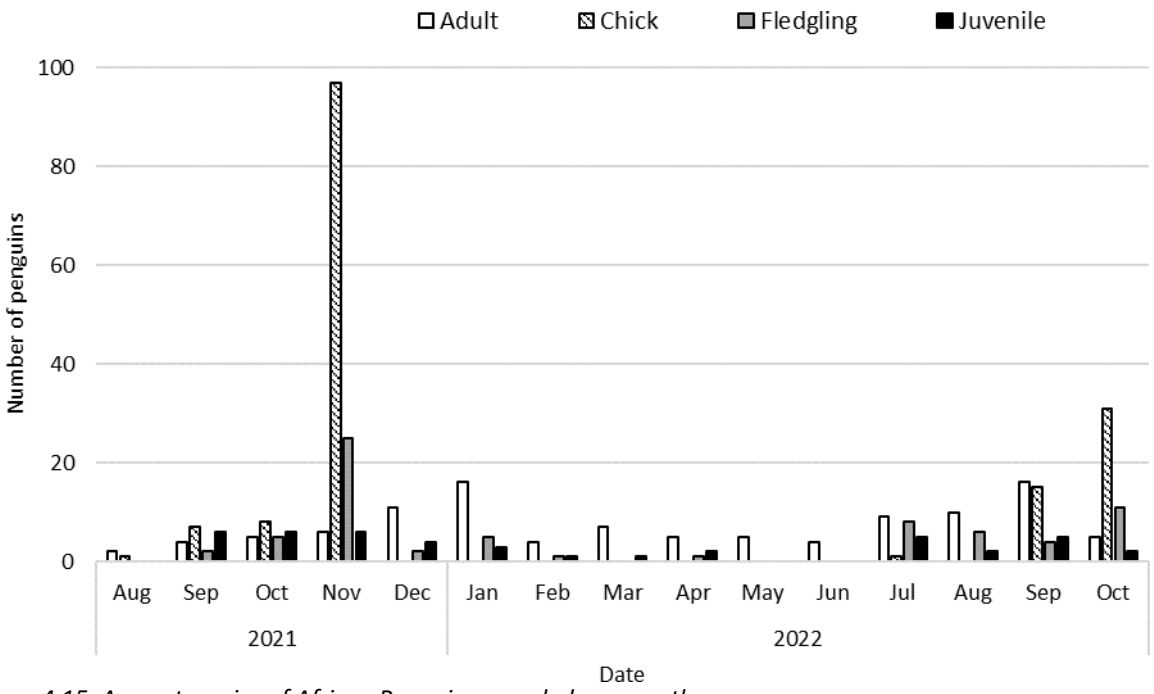


Figure 4.15: Age categories of African Penguins sampled per month

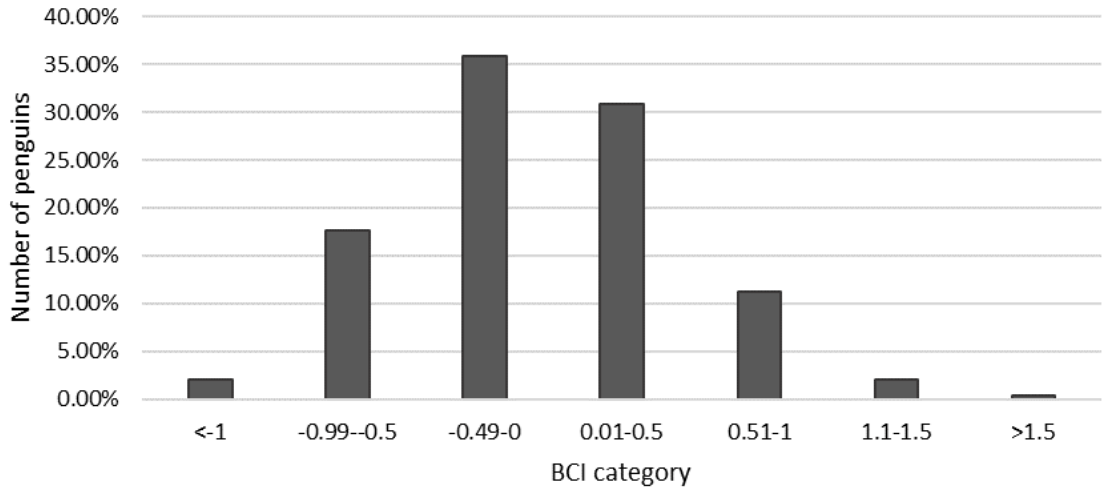


Figure 4.16: Frequency of different body condition indices (BCI) calculated for penguins admitted to SANCCOB

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Table 4.12: Origins and ages of penguins admitted to and sampled at SANCCOB

Location (NW to East) (colonies underlined)	Total admitted	Number sampled per age category				Total Sampled	Proportion sampled
		Adult	Juvenile	Fledgling	Chick		
Port Nolloth	2		2			2	1.00
Elands Bay	2		1			1	0.50
Lambert's Bay	9	3	4	1		8	0.89
Velddrif	21	8	2	4		14	0.67
West Coast National Park	5	1	1	2		4	0.80
Yzerfontein	4	1	1	1		3	0.75
Dassen Island	12		1	4	6	11	0.92
Melkbosstrand	12	1	1	5		7	0.58
Robben Island	**44**	6	1		12	19	0.43
Cape Town	14	2	3	4		9	0.64
Hout Bay	10	2	3	3		8	0.80
Cape Point	1	0	0	0	0	0	0
Simon's Town	**217**	47	14	23	29	113	0.52
Strand	6	1	1	3		5	0.83
Betty's Bay	**406**	35	8	13	79	135	0.33
Kleinmond	2	2				2	1.00
Hermanus	2			1		1	0.50
Dyer Island	**78**			6	34	40	0.51
Total	**847**	**109**	**43**	**70**	**160**	**382**	**0.46**

Table 4.13: Sexes of adult penguins admitted to SANCCOB, determined using the Farah discriminant function, and proportions sampled

Sex	Admitted	Sampled	Proportion sampled
Male	39	32	0.81
Female	55	41	0.75
Unknown	41	27	0.67
Total	**135**	**100**	

Table 4.14: Body condition indices of penguins admitted to and sampled at SANCCOB

BCI category	Admitted	Sampled	Proportion sampled
<-1	15	11	0.73
-0.99-(-0.5)	128	79	0.62
-0.49-0	261	128	0.49
0.01-0.5	225	93	0.41
0.51-1	82	43	0.52
1.1-1.5	15	6	0.40
>1.5	3	3	1.00
Unknown	118	19	0.16
Total	847	382	0.46

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3.3.2. rRT-PCR and NGS results

Fifteen penguins tested IAV-positive on samples taken soon after admission to SANCCOB. Two from November 2021 and September 2022 respectively tested negative on the subsequent H5 and HP H5 tests. The first sample was suspected to have been contaminated by another positive sample from a clinically ill cormorant, that was handled immediately beforehand, and the bird (a healthy P2 chick) and its pen mates tested negative when sampled three and six days later, respectively. The other sample was from an adult that only had a dislocated knee and both samples were classified as negative. Though not suspected to be a false positive at the time, another positive P4 chick from Stony Point (Betty's Bay), sampled on 18 November 2021, was underweight (BCI=-0.82) and required treatment for babesiosis, but no signs of HPAI were observed. It tested negative two weeks after admission and was released after another 3 weeks. It arrived with three other chicks that all tested negative. A partial genome sequence was obtained (A_African_penguin_ZA_669895_AP639_2021; Abolnik *et al.*, 2023) and the other bird that tested positive in the same batch (though sampled on a different day), was found to have a non-identical sequence (AP683: A/African penguin/South Africa/669895 AP638/2021; partial genome), so does not seem to be a source of contamination. However, there may have been contamination from infected Cape Cormorants, also admitted from Stony Point and sampled on the same day. They died or were euthanised and tested positive post-mortem (669572) so the samples taken when they were alive were not submitted, but may have been handled with the penguin sample. The post- mortem samples were not sequenced, however.
The virus obtained from Stony Point (whether from the penguin chick or Cape Cormorants), shared a recent common ancestor with some of the earliest viruses detected in seabirds in 2022 (Abolnik *et al.*, 2023).

Twelve penguins sampled at SANCCOB (3%) were deemed infected with HPAIV. Two of the twelve IAV-positive samples tested H5 PCR-negative at AssureCloud but HP H5 and N1 tests at ARC- OVR were positive and one of these yielded a complete clade 2.3.4.4b H5N1 HPAIV genome through NGS (A_African_penguin_ZA_698162_AP446_2022 (SA13); Abolnik *et al.*, 2023). Another eight tested HP H5 and N1 positive but NGS results are pending. Results of ARC-OVR testing are not available for the last two, but clade 2.3.4.4b H5N1 HPAI was confirmed by NGS from a partial genome for one (AP683: A/African penguin/South Africa/669895 AP638/2021), and clade 2.3.4.4b H5Nx HPAI for the other (only HA sequenced; A/African penguin/South Africa/673279 AP749/2021).

Of the twelve that tested HPAI H5N1-positive, one was a chick that died three days after admission, one adult and one juvenile recovered from mild clinical signs, one juvenile was euthanised after two weeks of treatment and nine were euthanised on admission to SANCCOB, based on severe clinical signs.

The first penguin identified as a true case was a P3 chick that arrived from Dyer Island on 23 November 2021 as part of a batch of 78 chicks removed on 20 and 23 November. Forty were tested, aiming to detect at least one case if 5% or more were infected. The PCR test positive proportion for the batch was 2.5% (0.1-12%). The chick apparently showed no obvious clinical signs on admission, and was noted to be in reasonable body condition (BCI 0.02), but it developed dehydration and signs of a severe respiratory infection two days later and died three days after admission. A partial genome was obtained (A/African_penguin/South Africa/669895/AP638/2021).
Because the chick had spent some days in the holding facility, SANCCOB decided to swab all the birds on 30 November and pool swabs where HPAI was not suspected. All tests were negative except for one pool, with a sample from another P4 chick from Dyer Island. It had tested negative on the admission samples taken on 23 November but became ill 7 days later, recumbent and breathing heavily and died. The rest of the birds that contributed to the pool remained healthy and were negative on a re-test. Interestingly, another chick from Dyer, that developed seizures a few days after admission, also tested negative.

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The second case was an adult from Stony Point, admitted on 22 December 2021, probably a male. He appeared to have moulted recently and was emaciated (BCI= -0.82) and seemingly had an eye infection. He was isolated after the positive test results were received and was recorded as having mildly increased breath sounds and sneezing. He tested negative 25 days after admission and was released a month later. However, the isolation with a dedicated carer caused him to become habituated, which may have been the cause for re-capture a week after he was released. Only an HA gene sequence could be obtained (A/African_penguin/South Africa/673279/AP749/2021). Serological testing was also done on a sample taken during recovery. High HI titres were detected: H5N8 1:512 and H5N6 1:256, consistent with a clade 2.3.4.4b H5 infection.

A juvenile that tested positive was admitted from Melkbosstrand (West Coast) on 11 August 2022. It was underweight (BCI=-0.33), with some apparent bile staining of the guano and regurgitated fluid on admission. He was observed to stand back on his hocks but was not suspected to be infected. The admission sample tested positive, however and was isolated until another test was performed. This was also positive so he was euthanised after 15 days to protect the rest of the birds in the centre.

An adult male (sex confirmed on PM) admitted from Strand on 14 August 2022 was suspected to be a HPAI case on admission and was euthanised immediately. The head was rolling back, it appeared overstimulated and to be foaming at the mouth. They area around the eyes was red and the bare patches above the eyes were slightly inflamed and seemed to be scratched. Body condition was recorded as thin and BCI was calculated as -0.31.

Of the twelve confirmed cases, eight were from the Simon's Town area between 3 and 21 September 2022. Seven were specifically from Foxy Beach at the Boulders colony and one from Muizenberg (10 km across the northwest side of False Bay). Six were adults, four males and two females (confirmed on PM), and two were juveniles. All were euthanised on admission due to suspected HPAI: one was simply unresponsive with no other obvious lesions but the others all had a neurological abnormality including ataxia, opisthotonus, tremors and twitches, braying and weakness. Three had reddened skin above the eyes, two with petechiation, and four had ocular exudate (serous or purulent). One appeared to have a swollen head, possibly due to sinusitis, and three had respiratory distress or difficulty. Two had exudate from the mouth. The six calculable BCI ranged from -0.65 to 0.06.

The complete genome (A/African penguin/South Africa/698162AP446/2022) was obtained from one of the adults admitted on 20 September 2022 and represented a cluster of viruses detected in, mostly dead, African Penguins from Simon's Town and Stony Point from September to November 2022.

One bird was suspected to be infected with HPAIV, due to mild head twitches, and was euthanised on admission, but tested PCR-negative.

3.3.3. Assessment of possible risk factors for HPAIV infection

3.3.3.1. Clinical signs associated with HPAI detection

Although two of the twelve cases had few clinical signs of HPAI, the highest odds ratios and most significant association with HPAI detection were related to the presence of neurological abnormalities ($P<0.001$) and abnormalities of the bare skin above the eyes ($P<0.001$) (Table 4.15). Three of the twelve birds had milder neurological signs such as head shaking, a slight head twitch and a head tilt, but tested positive for AIV. The presence of green guano and a swollen head were not significantly associated with virus detection, but the odds of a bird with ocular or respiratory abnormalities testing positive were increased and the association is slightly significant ($P=0.02$).

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Table 4.15: Selected clinical signs shown by penguins admitted to SANCCOB and the strength and significance of the association with HPAIV detection

Clinical sign	Odds ratio		P (mid-P exact)
	Estimate	95% CI	
Neurological abnormality	72	18-285	<0.001
Red, petechiated or injured bare skin above the eyes	37	9.3-145	<0.001
Ocular exudate or other abnormality in both eyes	12	2.2-67	0.02
Respiratory abnormality	7.4	1.8-30	0.02
Swollen head	17	1.4-198	0.09
Green guano	4	0.47-36	0.28

3.3.3.2. Location

Eight of twelve HPAI-positive birds originated from Simon's Town, with only two birds from other colonies: one adult from Stony Point (Bettys Bay) and one chick from the Dyer Island and the other two from sites away from colonies. The estimated upper 95% confidence limits for HPAI prevalence in the large groups of chicks removed after abandonment were at 6% for Stony Point (10% if the healthy chick is included), 12% for Dyer Island and 12% for Simon's Town (overall 4%).

3.3.3.3. Age

Of the twelve confirmed cases, 8 were adults (OR=5.3 (1.57; 18.08), P= 0.007; mid-P exact), 3 juveniles (OR=2.74 (0.715:10.58), P=0.18; mid-P exact) and 1 P3 chick (OR= 0.12 (0.015:0.944), P= 0.017).

3.3.3.4. Body condition

BCI could be calculated for ten of the twelve birds that tested positive for HPAIV. Eight had a BCI<0, and were therefore likely underweight (OR= 2.7 (0-13); P= 0.2 (Mid-P exact)).

3.3.3.5. Sex

Of eight positive adults where sex could be estimated, six were males. OR= 4.5 (0.84-24), P=0.08 (mid-P exact).

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4. Discussion

4.1. Overview

This study involved the sampling of 945 African Penguins in a 22 month period, from the two southernmost Namibian colonies and from all major South African colonies and when admitted for rehabilitation at a seabird centre in Cape Town. Penguins admitted for rehabilitation were found at locations ranging from as far north as Port Nolloth (500 km north of Cape Town), to Dyer Island, approximately 100km southeast of Cape Town.

No avian influenza virus was detected in any penguin sampled in a breeding colony, though serology in South African birds provided evidence of previous avian influenza infection in eight birds, five of them possibly due to clade 2.3.4.4 H5 HPAI. The only AIV detections, which were confirmed to be of clade 2.3.4.4b HPAI H5N1 viruses, were in twelve penguins admitted for rehabilitation.

4.2. Findings and observations after sampling in African Penguin colonies

4.2.1. Lack of avian influenza virus detection in penguin colonies

African Penguins sampled in the wild were restricted to adults found in and around nests, and they generally appeared healthy, with few signs of disease and normal body condition. These samples could not provide evidence that AIV was circulating in these colonies at an overall prevalence of more than approximately 1.8%, during this time. The estimated upper 95% confidence limit was however higher for individual colonies and up to 11% on Dyer Island, which had the smallest sample size. Estimated upper limits for other sampling trips, at other times, were as high as 35% for Stony Point (Betty's Bay) in January 2022 and 13% at Bird Island in May, dictated by sample sizes aimed at achieving the overall goal of 382. However, assuming random sampling was somehow achieved, sampling on Halifax Island in Namibia should have produced at least one positive PCR test if virus was present as more than approximately 3% prevalence in the healthy adult birds sampled and on Possession Island if there was an approximate prevalence of 6% or more. No such claims can be made for younger penguins and those not frequenting the nesting areas, but it unlikely that there is long-term maintenance or high levels of avian influenza virus in adult African Penguins. Further indications of the penguins' health was provided by an absence of penguin circovirus and of avian paramyxovirus serotype-1 antibodies.

The sampling period was protracted (seventeen months from January 2021 to May 2022), which is not ideal for a cross- sectional study, but it was dictated by logistical factors such as limited peak breeding seasons, personnel availability and distance to colonies. However, 59% (n =160) of Western Cape samples were taken in a six-week period from July to September 2021 and were well-distributed across the sampled colonies, which only excluded Dassen Island (Table 4.7). This improves the quality of the survey as "cross-sectional" but it also means that most sampling was done before the peak of the HPAI (H5N1) seabird outbreak, in October and November, and no sampling was done during this time. Additionally, the sampling on Dyer Island, where most penguin deaths occurred, was completed in August, the month with lowest overall suspected cases. It was also not possible to sample randomly, as assumed by the sample size calculations and calculation of upper confidence intervals for prevalence estimates. This was unavoidable, however, and the best that could be done was to avoid convenience sampling. The estimates of maximum prevalence, if not precise, can still be used to compare results of sampling at different times, colonies and groups and to indicate the level of confidence in the results.

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4.2.2. Avian influenza serological findings from penguin colonies

Serology provided some evidence for previous IAV infection and survival after HPAI infection, which contrasts with the findings of Parsons *et al.* (2016). They found no antibodies against H5 or H7 avian influenza in 439 samples collected in South Africa and Namibia from 2007 to 2013, even though there were LPAI viruses known to be present and HPAI viruses in ostriches in some years. The HPAI ostrich viruses were only detected in ostriches (Abolnik *et al.*, 2010), however, and it's possible that there is little circulation of LPAI viruses in African Penguins. Other surveys also failed to find avian influenza antibodies in the other *Spheniscus* species, in South America (Smith *et al.*, 2008; Uhart *et al.*, 2020; Travis *et al.*, 2006), though sample sizes were limited. The poor specificity (94%) of the ELISA test, determined in other bird species, may mean that the three positive tests are not actually an indication of previous avian influenza infection. However, the two positive samples from Dassen Island in January 2021, that came from birds only about 20m apart, suggests there may have been infection there, either with local transmission or from a common source. The single positive ELISA result for Stony Point from January 2022 is possibly more questionable. The associated negative HI tests suggest these infections were not due to a clade 2.3.4.4. H5 virus, and likely not another H5 virus but possibly to some other serotype.

The five serum samples with HI titres, including titres <16, have been interpreted as possible indications of previous clade 2.3.4.4. H5 infection, despite the negative ELISA tests, because of apparent poor sensitivity demonstrated by the ELISA test during a vaccine trial in African Penguins. It was found that 36% of positive (≥16) HI tests, on samples from birds that received an inactivated whole virus clade 2.3.4.4b HPAI H5N8 vaccine, were associated with negative ELISA tests. 86% of the associated HI titres were 16 and 32 (maximum 128). However, the positive (>16 titres) H5 HI test from Dassen Island in January 2021 is then surprising, given that H5N8 had last been detected in June 2018 and H5N1 was not detected in the Western Cape until May 2021. Possibly, the H5N8 virus circulated longer that was thought, or the antibody lifespan is longer than expected. Alternatively, the H5N1 viruses were introduced earlier, before mortalities were noticed. This may be credible, given the estimation by Abolnik *et al.* (2023) that the progenitor of the initially-detected virus arose between December 2020 and March 2021. The contrasting lack of antibody detection in January 2022 may count against this theory, however, assuming penguins were more likely to become infected and seropositive as case numbers rose elsewhere. However, fewer samples were taken in 2022 and were therefore less likely to detect a low antibody prevalence. Additionally, no positive or suspected cases of HPAI in penguins were reported from Dassen Island in 2021 or 2022 which is more evidence against a higher prevalence.

The positive and suspect tests on Bird Island in May 2021 are slightly more consistent with virus detections elsewhere and the low titres on Dyer and Robben Island in August 2021, as the mortality rates in seabirds were about to pick up, may be a sign of early seroconversion, or evidence of earlier infection, before the first detection in a penguin from Simon's Town.

The low proportion of avian influenza seropositive samples does not provide any evidence of significant population-level humoral immunity. The penguins may have been exposed previously, nonetheless, and either have undetectable antibody levels or cellular immunity. This may still protect against future infection with homologous viruses, but the lack of evidence for robust herd immunity is concerning.

4.2.3. Characteristics of penguins sampled in colonies

Of the six seropositive birds where sex could be determined, five were females, and the odds were therefore five times higher for a female to test positive, than for a male. The association was not statistically significant, possibly due to the low number of avian influenza seropositive samples. This

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should be investigated further, however, bearing in mind that this may indicate that females are more resistant to HPAI, as they survived to develop antibodies.

A possible male sampling bias was detected in Namibia, and, to a lesser extent, in Simon's Town. The cause in Simon's Town may be explained by higher proportion of "loafing" males that were sampled. Though why more loafing males would be selected or why more males would be alone than females is not clear. It is possible that females spend more time at sea, and are less likely to be found in colonies. Though not statistically significant, Spelt and Pichegru (2017) found that female African Penguins spent slightly more time off the nest and that female penguins' hunting trip duration increased with the number of chicks. However, a brief analysis of sex and chick number for the Namibian penguins that were sampled (not included in results) showed a similar proportion of females with one chick to those with two. Females spending less time in the colony may also explain the possible "resistance" and higher proportion of seropositive samples from females. If they are exposed to lower doses of virus, they perhaps are not so severely affected and are more likely to survive.

The Farah discriminant function was not derived with data from Namibian penguins, though birds from overseas zoos were included, so it is possible that the function misclassifies some female Namibian penguins, if they are generally bigger than South African females. However, Duffy (1987) compared beak sizes between Namibian and South African colonies and the only significant difference identified was between beak length at Mercury and Dyer Islands. An average difference of 1.3mm in both beak dimensions, between males and females in South Africa and Namibia, can be calculated from his data, but subtracting 1.3mm from all Namibian beak measurements in this study only reduces the number of males by two and adds one female. Unless Namibian penguins have been intensively selected for bigger heads in the last thirty years, it seems unlikely that the Namibian penguins are different enough to affect the estimated sex ratio so drastically. The theory that the depleted sardine (*Sardinops sagax)* and anchovy (*Engraulus encrasicolus)* stocks have forced penguins to eat larger fish, and favoured females with bigger beaks, was briefly considered. However, the bearded/ pelagic goby (*Sufflogobius bibarbatus*), that now seems to be an important prey item (Kemper, 2015), is a similar size. Measurement bias was also considered but the methods used were the same as those described in Campbell *et al.* (2016) and if an incorrect method was used, it was used by all vets performing the measurements.

The theory that the Namibian population is deficient in females was then considered as a tempting explanation of the drastic drop in breeding numbers. Spelt and Pichegru (2017) found a higher proportion of male chicks on Bird Island and describe how other seabird species produce more male chicks under good environmental conditions. However, the bias in this study extended to birds found with chicks (on Halifax Island at least) that must therefore have had a breeding partner, which implies that the source was somehow from sampling and not the population. On Halifax Island, the birds were selected very differently from anywhere else, which may offer an explanation there, though not for Possession Island. The birds nest in the open, in dense groups, on Halifax and were captured from the edge of the group. Though it is unlikely that handlers selected bigger and more aggressive males from the edges of the colony, it is possible that the males were less likely to withdraw into the colony and were more easily accessed.

The lack of sampling of birds in the centre of the colony on Halifax is also a source of bias, especially when considering that avian influenza would transmit more easily there. But the birds in the centre must walk out past those at the edge and it seems unlikely that all at the edge would escape infection, if the virus was actively circulating at a significant level.

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4.3. Findings from avian influenza PCR testing at SANCCOB

The lack of AIV or antibody detection in wild healthy penguins, and the detections only in birds admitted for rehabilitation, mostly in adults, suggests that the prevalence is generally low, but that the virus causes severe illness in African Penguins, especially in adults. The Simon's Town area also appeared to be a hotspot. However, the inherent bias in sampling penguins admitted to a rehabilitation centre and the exclusion of small chicks should be considered when interpreting the findings. Admissions rely heavily on the level and consistency of monitoring in the colonies and on the consistency of visitors to other areas of the coast, especially more remote locations.

Penguins sampled at the rehabilitation centre therefore do not provide an accurate estimate of population prevalence, except possibly where large batches of relatively healthy chicks were cleared from the colony, but can be used to explore possible risk factors and to obtain virus for molecular analysis and phylogenetic investigations. Given the lack of detection in healthy adults in the colonies, the sampling at SANCCOB was a valuable complementary source of data targeting adults with compromised health, and for younger birds. Additionally, chicks abandoned by their parents provided large enough sample sizes to estimate a maximum prevalence of 12% among (older) chicks and fledglings at Stony Point and Dyer Island in November 2021 and at Simon's Town in October 2022.

The one case in a chick was detected on admission from Dyer Island, the worst-affected site in 2021. There had already been over 13 000 deaths in Cape Cormorants on the island, attributed to HPAI, though the mortality rate had decreased, and 86 suspected cases in African Penguins had been counted since 11 October. The chick may have been showing few signs initially, but more subtle signs may have also been missed or not recorded in the rush to admit large batches. Nonetheless, the bird became severely ill within three days and died, suggesting a fatal infection. The other chick, that tested negative on admission, but became ill a week later, also showed respiratory signs. However, these are common in penguin chicks with compromised health, so may not be a useful indicator of possible HPAI infection. The latter case seems to have had a rather long incubation period to have been infected in the colony, but there was no other evidence of transmission after admission. From these cases and lack of detection in other chicks, especially after the whole holding facility was sampled in late November 2021, subclinical virus circulation in the centre seems unlikely. A true infection of the healthy chick from Stony Point would count against that theory, however. It is impossible to tell if the chick was really infected or if the sample was indeed contaminated. Contamination seems to have been possible, but it was only the lack of clinical signs that sparked the retrospective investigation into the validity of the test result.

The adult penguin from Stony Point (December 2021) and the juvenile from Melkbosstrand in August 2022 demonstrated that mild clinical infections and recovery are possible, at least in older birds. There were no other confirmed penguin cases reported from Stony Point in 2021 and most adults and juveniles were tested, so perhaps the prevalence was low, or infections less severe for some reason. There were few deaths in Cape Cormorants at Stony Point at that time and relatively fewer overall at Stony Point compared to Dyer Island. The environmental viral load and dose were perhaps lower, therefore. Both these birds were underweight, which may have made them more susceptible to infection and disease. However, though infected birds seemed slightly more likely to be underweight, the association was not statistically significant. Possibly, they were only found and tested because of their poor body condition. A limitation of cross-sectional studies is that cause and effect cannot always be assigned with confidence, especially when it comes to serological findings or subclinical or chronic disease. Some characteristics may have contributed to survival (and being alive and available to study), rather than infection (Dohoo *et al.*, 2014).

All nine positive birds admitted alive from the Simon's Town/ False Bay area (including the bird from Strand on the opposite side of the bay) in 2022 showed severe clinical signs, and neurological

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abnormalities and pathology of the eye skin were strongly associated with HPAI infection. These signs can be useful indicators of this HPAIV H5N1 infection in African Penguins, especially where laboratory confirmation is not possible. Sampling was biased towards Simon's Town penguins, partially because of the relatively high numbers admitted with suspected HPAI in September 2022, 75% of which were sampled.

The apparent larger number of female penguin admitted to SANCCOB is consistent with the findings of Pichegru and Parsons (2014), who published on the bias towards juvenile and adult female African Penguins that died after admission to SANCCOB, between 2004 and 2012. They found that the cause of death was usually starvation, parasitism or disease and postulate that the smaller females are less resilient in poor environmental conditions. They reference multiple other studies that also recorded female-biased mortality in other penguin species. However, despite an apparent female admissions bias, the data from this study hints that the odds of a male having HPAI was higher, though the association was not statistically significant.

Looking purely at test results and the sampling bias towards older birds, birds in poorer body condition and birds from Simon's Town with HPAI signs, it seems possible that cases may have been missed in chicks, especially younger ones, and maybe in some healthier-looking birds. Records of clinical examination were also scarcer for chicks admitted in big batches. This could be because most had few clinical signs, or because the admission was rushed and the birds were less carefully examined. However, the sampling of all birds in the holding facility after the infected chick from Dyer Island was diagnosed in November 2021, and the lack of evidence for virus spread in the facilities in general, tends to suggest that the sampling bias did not play a major role. It would be ideal to sample more chicks in future outbreaks (maybe with smaller swabs), however, to confirm their susceptibility.

4.4. Logistics of African Penguin sampling in colonies

The study was planned in 2019 but only started in 2021 due to the time required to source funding, to negotiate acceptable sample sizes and sampling protocols with permitting conservation authorities and to procure the necessary equipment and consumables. All of these were prolonged to some extent by the COVID-19 pandemic. Few other disease studies have been conducted recently in South African seabirds, and it seemed that the sample sizes calculated for this study appeared very large to the permitting authorities. Other studies, such as those involving GPS tracking, involve far fewer birds, probably due to the high cost of the tracking devices. Permits were only issued after the South African colony sample size was halved, a time limit was set on handling, additional samples were planned, sampling teams were limited to three people and monitoring of disturbance caused by sampling was planned for Bird Island. It was also agreed that all birds handled would be microchipped, to contribute to another long-term project and collaboration with other researchers would be attempted as much as possible. Procurement also caused delays as it was hoped that administration staff would be able to order from lists of required items, but more technical knowledge was required for interaction with potential suppliers.

Working on islands meant that transport was dependant on boats, and subject to weather conditions. Plans had to be flexible enough to accommodate weather changes, and this limited field assistants to those with flexible work arrangements. Given the penguin handling expertise required, this limited available field assistants to a very small pool. Arranging safe and affordable transport by boat was always challenging and was a major limiting factor in Namibia, where only the unreliable government research vessel travels to most seabird islands. Transport challenges, the penguin population crash and the inability to remove samples from the country for testing, as well as challenges with laboratory capacity, severely limited productivity in Namibia.

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Some of the islands have their own power supply, but refrigerators are invariably unreliable, so a camping fridge/ freezer with a built-in thermostat was indispensable and 12 volt batteries, solar panels and an inverter were needed on Bird Island, for running the fridge and centrifuges. Processing 20ml of blood per bird, staining blood smears and reading haematocrit and serum protein from microhaematocrit tubes was very time-consuming. For future projects, it therefore would be ideal to have at least one extra field assistant to process samples while the rest of the team continues sampling. Birds sampled per day varied with island and colony conditions, with faster sampling in denser colonies. This had to be factored in when budgeting time for each island, and experienced seabird researchers were consulted beforehand.

5. Conclusions

This study found no avian influenza virus in healthy wild African Penguins, sampled across their breeding range, and therefore no evidence that they play a major role in avian influenza epidemiology. There was serological evidence of possible previous infection at some South African colonies, but antibodies were only detected in a small proportion of birds sampled. Sampling of penguins admitted for rehabilitation provided an opportunity for virus detection in debilitated birds and the younger age categories.

Though mild infections were detected, the majority of virus detections were in severely ill adults from Simon's Town, during an outbreak. The neurological abnormalities observed and pathology of the bare skin above the eyes were found to be strongly associated with virus detection. Fewer infected chicks were admitted, though they also came from a colony with an outbreak. They displayed only respiratory abnormalities and sampling may have been insufficient to detect such cases. Further work to investigate the susceptibility of chicks, and also a potential association with sex, is recommended.

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Chapter 5

Vaccination of African penguins (Spheniscus demersus) against clade 2.3.4.4 H5 high pathogenicity avian influenza

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1. Introduction

High pathogenicity avian influenza (HPAI) has long been recognised as a danger to poultry health but clade 2.3.4.4b H5 viruses have also become a conservation threat, killing thousands of wild birds on nearly all continents (CMS FAO Co-convened Scientific Task Force on Avian Influenza and Wild Birds, 2023). At least a thousand endangered African Penguins (*Spheniscus demersus)* have died in southern Africa (Abolnik *et al.*, 2023; Roberts *et al.*, 2023; Umberto *et al.*, 2020) but options available to manage the disease are limited (Roberts *et al.*, 2023).

Historically, vaccination of poultry against HPAI has been restricted to a few countries (Swayne and Sims, 2021), and vaccination of wild species, including *Spheniscus spp*, has been done only in zoos (Bertelsen *et al.*, 2007; Furger *et al.*, 2008; Lécu *et al.*, 2009; Philippa *et al.*, 2007, 2005). Vaccination of wildlife in general is limited by the logistical challenges of ensuring sufficient doses and coverage of populations over which humans exert relatively little control (Barnett and Civitello, 2020). Ensuring vaccine efficacy under natural conditions can also be difficult (Maichak *et al.*, 2017). Vaccination of domestic birds against HPAI has been limited, firstly because a stamping out approach was deemed sufficient for control of many previous HPAI viruses (Swayne *et al.*, 2011), but also because AI vaccines, though protecting against disease, do not completely prevent infection, shedding of virus and further transmission. Subclinical infections make disease surveillance more expensive and complicated, but proof of disease freedom is often required for trade in animal products and vaccination is a common trade barrier. Vaccines that allow continued virus circulation may also drive increased viral virulence (Barnett and Civitello, 2020). Despite these challenges, control of HPAI, especially the clade 2.3.4.4b viruses, without vaccination is becoming increasingly impossible and guidelines for responsible vaccine use have been shared (Swayne and Sims, 2023).

Vaccination may be necessary in future to maintain functional seabird populations, but current research is insufficient to justify the expense and effort that would be required to vaccinate wild penguins or other threatened species. The most widely used avian influenza vaccines comprise inactivated whole viruses but their application to wild birds seems unrealistic. They are relatively inexpensive to produce but require virus propagation to high antigenic titres in embryonating chicken eggs, parenteral administration and usually two or more doses (Swayne *et al.*, 2014). Their production is slow (Swayne and Sims, 2021) and serological differentiation of infected and vaccinated animals (DIVA) is not possible unless the vaccine contains a neuraminidase (NA) type antigen heterologous to the circulating field virus and is used in conjunction with an NA antibody test.

In vitro-produced recombinant antigen vaccines, synthesised in plant leaf tissue, insect or bacterial cells, offer alternatives to conventional inactivated vaccines (Balke and Zeltins, 2020; Swayne *et al.*, 2014). Rapid synthesis of many doses is possible once a field virus has been genetically sequenced and no live virus is required (Abolnik *et al.*, 2022b). The haemagglutinin protein expressed on a virus-like particle (VLP) is highly ordered in a dense array, similar to a live virus, which stimulates a strong cellular and humoral response (D'Aoust *et al.*, 2010). The absence of internal virus proteins, like the nucleoprotein (NP), allows for DIVA using standard ELISA tests.

Antibodies to the haemagglutinin (HA) protein are considered responsible for the majority of virus binding, and therefore for protection against HPAI (Swayne and Sims, 2021; Van Der Goot *et al.*, 2005). The haemagglutination inhibition (HI) test detects antibodies to HA protein and so is a correlate of protection against AIV and can be used to assess the immunogenicity of AI vaccines. The recommendation for AI vaccines for chickens in the WOAH Terrestrial Manual (WOAH, 2021) states that a vaccine should evoke minimum HI serological titres of "1/32 to protect from mortality or greater than 1/128 to provide a reduction in challenge virus replication & shedding". Work by Kumar *et al.* (2007), Lee *et al.* (2004), Maas *et al.* (2009), Sasaki *et al.* (2009) and Swayne *et al.* (2006) supports these cut-offs approximately: variation may be associated with the homology between the vaccine

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and challenge viruses studied and the quantity of antigen used in the HI test. There are unfortunately no published recommendations to be found for protective antibody levels in non-poultry species. Despite the value of using HI tests to assess vaccine immunogenicity, low HI levels may not necessarily denote a poor level of protection (Swayne and Sims, 2021), especially against heterologous virus strains (Spackman *et al.*, 2014; Swayne *et al.*, 2015). This may be due to cell-mediated or mucosal (IgA) immunity or humoral immunity from non-HI antibody immunity. Correlates of protection for these immune responses still need development (Calzas and Chevalier, 2019).

This study was designed to assist with the decision as to whether vaccination of African Penguins may be justified and also in the choice of vaccine, vaccine formulation and dosing schedule required. Previous experience of avian influenza vaccination in penguins, especially African penguins, is very limited. Only five peer-reviewed publications have included penguins and these included only 22 African penguins. These were birds vaccinated in zoos, with inactivated vaccines (Bertelsen *et al.*, 2007; Furger *et al.*, 2008; Lécu *et al.*, 2009; Philippa *et al.*, 2007, 2005). The studies all assessed antibody levels at 4-8 weeks after a second dose of vaccine that was administered at 6 weeks after the initial vaccination in four studies and at 5 weeks in one study. All researchers except Bertelsen et al, used homologous HI antigens and all reported geometric mean antibody titres (GMT) except Lécu *et al.* (2009). They report only "mean" titres, so this study has been excluded when comparing GMT. The other four studies found that GMT in the penguins, at 4-8 weeks after a second dose, were between 1/22 and 1/119 (log2 titres 4, 6, 6, 7 respectively). Where an overall geometric mean titre (GMT) was available (three studies), penguin titres were one order of magnitude lower than the average for two studies (Philippa *et al.*, 2007, 2005) and for Bertelsen *et al.*, they were 3 orders of magnitude lower (4 vs 7). Four of the five studies considered the antibody levels protective at 4-8 weeks after the second vaccination (above a titre of 32 in two studies and 40 in the other two). However, two of these four studies also sampled at 180 days and the antibody levels had already dropped drastically, below protective levels.

Osterhaus *et al.* (2007) compiled a report on the vaccination of over 40 000 birds against H5 and H7 AI in zoos in thirteen EU member states, using commercially-available inactivated, water-in-oil adjuvanted H5 vaccines. Data from Philippa et al and possibly from Lécu *et al.* were incorporated. Antibody titres, determined by HI tests, were measured at 21-40 days after the second vaccination. Overall, only 49% of birds had titres ≥1/32 after one vaccine dose, though two vaccines performed better than the others, and some orders responded better (e.g. Anseriformes, Ciconiiformes, Phoenicopteriformes). After a second dose, the overall GMT was 89, and 76% had titres ≥1/32. Sphenisciformes responded well to one of the vaccines, formulated with an H5N2 virus (A/duck/Pottsdam/1402/86). This can be attributed to 31 Humboldt Penguins, vaccinated subcutaneously, that had a post-vaccination GMT of 1/131, with 94% of birds with titres ≥1/32. Consolidated data from 73 unidentified penguins, that received the same vaccine, averaged a much lower GMT of 1/26. There may therefore be variation between penguin species, but there is also limited variation on the other variables that may have affected the HI test results.

Besides the efficacy of a vaccine, the other major concern is safety and the frequency of adverse reactions. Furger *et al.* (2008) reported minor injection site swelling in a peacock but no other adverse reactions in the other 364 birds, which included 40 Humboldt Penguins (*Spheniscus humboldti*). Philippa *et al.* (2007) vaccinated over 3000 birds and reported five mortalities due to catching and handling stress but only one mortality was attributed directly to the vaccination. This was a penguin with a subcutaneous abscess at the injection site. Lécu *et al.* (2009) reported local reactions in three Anseriformes and an ibis, and systemic reactions in 17/4369 birds. These birds showed apathy, weakness and anorexia. Osterhaus *et al.* (2007) stated that 0.04% of birds had local vaccine reactions and 0.015% had general reactions. Most were ratites, with local reactions, that had received large volumes of vaccine subcutaneously (5 or 10ml) and some birds were lame after intramuscular injection. Total mortality due to handling and stress was 0.5% and higher incidence in some zoos

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highlighted the importance of skilled handlers. Philippa *et al.* (2005) and Bertelsen *et al.* (2007) considered the vaccines safe and did not link them or their administration to any deaths that took place during the study period.

This chapter describes a vaccine trial in African Penguins, aimed at assessing and comparing the magnitude and duration of antibody response induced by two clade 2.3.4.4b H5 HPAI vaccines, administered with and without a second dose. Twenty-four captive African penguins were divided into four treatment groups that received either an inactivated whole virus vaccine or a plant-derived virus-like particle (VLP) vaccine, each on two different dosing schedules. The magnitude and duration of antibody response were compared.

2. Objectives

1. Measure and compare the magnitude of immunogenicity of two HPAI Clade 2.3.4.4 H5 vaccines in African Penguins: a plant-produced clade 2.3.4.4 H5 virus-like particle vaccine and an inactivated whole virus clade 2.3.4.4 H5 vaccine.
2. Measure and compare the duration of protective antibody response of the two H5 vaccines in African Penguins, up to a maximum of 12 months.
3. Assess the need for a second (booster) vaccination against HPAI Clade 2.3.4.4 H5 to achieve protective antibody levels in African Penguins.
4. Assess the safety of the two H5 vaccines in African Penguins.

3. Materials and methods

3.1. Vaccine production

The methods described by Abolnik *et al.* (2022) were used to produce clade 2.3.4.4 H5 virus-like particles transiently in modified tobacco plant (*Nicotiana benthamiana*) leaves. The H5 was expressed from a codon-optimized DNA sequence based on South African field strain A/Speckled pigeon/08-004B/2017 (H5N8) (accession number AVV60712). Leaves were infiltrated with the recombinant *Agrobacterium tumefaciens* vectors containing plasmids with the H5 DNA sequence.
After purification, VLPs were diluted in phosphate-buffered saline (PBS), pH approximately 7, with 5% trehalose as stabiliser. This was formulated into a vaccine, on the day of administration, by the addition of a commercial mineral oil adjuvant, Montanide™ ISA 71 VG (Seppic, Paris, France) in a 50:50 ratio.

Deltamune Animal Health Solutions (Pty) Ltd produced a small batch of their standard closely related South African clade 2.3.4.4 H5N8 virus (A/chicken/South Africa/Villiers/2017; HA protein accession number AVV60593). The virus was isolated at Deltamune and it is the seed strain for the standardized antigen used for HI testing by accredited veterinary laboratories. The egg-grown virus was chemically inactivated and safety tested by back-inoculation as per standard procedures and was also adjuvanted with Montanide™ ISA 71 VG.

Given the relatively low antibody response measured in penguins by previous researchers, a dose of approximately 500 HAU (Inactivated virus: 512 HAU, VLP: 500 HAU) was used in this study. This aimed also to compensate for a body mass of 2.5 to 3kg, compared to the much lower body mass of young chickens

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3.2. Experimental design

3.2.1. Sample size calculation

The sample size calculation for comparing two means with unequal variances was used. The calculation was performed using the sample size calculator available online from EpiTools (Sergeant, 2018).

Initially, the sample size was calculated to estimate the mean antibody titre in each group, based on the smallest estimated difference between groups, expected to be the difference between mean $\log_2$ antibody titres before and after the booster vaccination with the VLP vaccine (a difference of 1.3, (Smith *et al.*, 2019). Basing the calculation on the most similar groups allows the sample size to be large enough for all other comparisons. This resulted in a sample size of 11 penguins per group (44 overall), which would require a request to also use penguins at the SANCCOB Gqeberha (Port Elizabeth) Centre. This was deemed impractical and a different approach was taken to reduce sample size.

It was decided to rather aim to determine whether there was a biologically significant difference between the mean titres in each group. This minimises sample size, compared to simply trying to detect a statistically significant difference. It was determined that a biologically significant difference in immunogenicity would only be concluded if a minimum difference in mean $\log_2$ titres of three could be detected.

To detect a mean $\log_2$ titre difference (effect size) of three, with 80% power, between two treatment groups with equal variances of 2.5, (based on Smith et al (2019)) five penguins are required per group (total of 20). We increased this number to 6 per group (total of 24) to allow for birds that may not be completely healthy, may be nesting or were found to be unusually stressed by sampling and may not be able to be sampled every time.

3.2.2. Trial subjects

Twenty-four healthy, adult African penguins, which were permanent residents in the “Home Pen” at the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB) Table View seabird rehabilitation centre, were the subjects of the vaccination trial. The penguins could not be released into the wild due to various physical and behavioural abnormalities.

Resident penguins at SANCCOB are kept in a sand and gravel-floored pen dedicated to resident birds, with a swimming pool and nesting boxes. The pen is surrounded by metal mesh and covered overhead with shade netting, along with the rest of the facility. The pen is accessed through a double gate system from the rehabilitation area, which is accessed only by staff, interns and volunteers. The centre is itself accessed through a double gate system with access control and mosquito-proofing. The penguins were housed and cared for as usual at SANCCOB during the trial. They are fed daily on fish and any behavioural abnormalities are noted by staff at feeding. Health problems are reported to the resident SANCCOB veterinarian. Resident birds are clearly marked as such with two different flipper bands (an SB (stud book) number and a name) and are also identifiable via microchips.

Thirty-six penguins were resident in the Home Pen at the time of the study. Eight birds older than 10 years were excluded, due to possible risk to geriatric birds and altered physiology and immunity. Another bird was excluded due to a chronic respiratory condition, due to both risk to the animal and a possible altered immune state that could bias the results. A tenth bird was excluded for behavioural abnormalities that were expected to complicate pre- and post-handling monitoring. Two more birds were excluded from the main trial due to pododermatitis (bumblefoot) but had recovered sufficiently in time to be used for a pilot trial.

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The study birds included 13 males and 11 females and their ages were estimated to be between 4 and 10 years. The ages of those that arrived at SANCCOB as adults are more uncertain. Most recent body masses were between 2.5 and 4.6 kg.

3.2.3. Randomisation and blinding

The penguins selected for the study were randomly allocated to four treatment groups, with six birds in each group. Approximately equal numbers of males and females were added to each group.
The birds were each assigned a random number using the Microsoft Excel random number generator function (=RAND()). The birds were sorted from highest to lowest random number and the females were assigned, in turn, to treatment groups, in order (1 to 4). The males were then assigned in the same way, starting with the group where the females had left off. Half of each group comprised males, except for Group 2b which had four males and two females.

Double blinding of the handlers who monitored the penguins daily, and the investigator who performed the data analysis, was incorporated initially (Annex 3.1) but was not maintained when some birds became ill.

In order to blind the handlers and the principal investigator, the treatment (vaccine and dosing schedule) applied to each group was not predetermined or visible at administration. A random number was allocated to each treatment, in an Excel spreadsheet supplied to the monitor, appointed as required by the South African Health Products Regulatory Authority (SAHPRA), and SANCCOB veterinarian, and the order of the random numbers determined the title of the group (1-4). The treatment assigned to each group was to be kept by the monitor in the spreadsheet until the data analysis was finalised. The syringes containing the vaccine were labelled out of sight of the principal investigator and handlers, and labelled only with the treatment group number. The label hid any visible vaccine (the VLP vaccine is greenish and the inactivated vaccine is more white).

3.2.4. Vaccination schedule

Planned vaccination schedule:
Two groups would receive the inactivated whole virus H5N8 vaccine. One of these two groups would only receive one vaccination and the other a second vaccination (booster) 4 weeks after the initial vaccination. At this time, the first group would be injected with adjuvant only, to eliminate any confounding effects of the physical injection and adjuvant, and also to maintain the blinding.
The other two treatment groups would receive the plant-derived H5 VLP vaccine. One of these groups would receive only one vaccination and the other would receive a booster (Table 5.1).

Revised schedule:
Due to illness in the VLP-vaccinated birds, the booster vaccination in the VLP group was cancelled and the booster for half of the inactivated vaccine group (n=6) was delayed until day 56.

Vaccination consisted of injection of 0.25ml vaccine intramuscularly in the pectoral muscle, using a new, sharp and sterile needle.

Table 5.1: HPAI H5 Vaccination regimen randomly allocated to four groups of African Penguins

Vaccination Regimen	**Group designation**
VLP vaccination: Day 0	1a
VLP vaccination: Day 0 and day 28 (56)	1b
Inactivated whole H5N8 virus vaccination: Day 0	2a
Inactivated whole H5N8 virus vaccination: Day 0 and day 28 (56)	2b

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3.2.5. Sampling schedule

Blood samples were taken on the day of first vaccination, to measure baseline antibody titres in serum, and then every two weeks for 8 weeks, and every two months thereafter for up to a year after vaccination (2 weeks after the first vaccination, at the time of the second vaccination of Groups 2 and 3 (day 28), day 42, day 56 and every 2 months thereafter for a maximum of a year) or until antibodies were no longer detected from two successive sampling sessions. Each bird was therefore sampled a maximum of 10 times.

Illness in the VLP-vaccinated group disrupted the program and though the first three samples were taken as planned, the fourth sampling was delayed to day 56, when the booster inactivated vaccine was given, and sampling was done twice more at 2-weekly intervals before the original schedule resumed (Fig. 1,Table 5.2).

Table 5.2: Sample sizes per sampling event- African Penguins vaccinated with an H5 avian influenza virus-like particle (VLP) vaccine (Group 1) and one (Group 2a) or two (Group 2b) doses of an inactivated high pathogenicity avian influenza H5N8 vaccine

	Days after primary vaccination										
	0	14	28	56	70	84	112	175	224	287	430
Group 1	12	11	5	0	0	0	0	0	0	0	1
Group 2a	5	6	6	4	6	5	5	5	6	0	0
Group 2b	6	6	6	6	6	4	5	6	6	6	0

3.2.6. Vaccination and sampling procedure

An SOP, included as Annex 3.2, was followed. The birds were handled before feeding, to prevent regurgitation. They were removed from Home Pen and placed in small temporary pens before and after handling.

The veterinarian performing the vaccination and (or) sampling was assisted by an experienced penguin handler (a SANCCOB staff member), who restrained the bird according to documented SANCCOB protocols. Correct bird identification was ensured by checking both flipper bands and the microchip number.

The vet conducted a clinical examination to measure heart rate and assess breathing and chest sounds and mucous membrane colour, mouth, eyes, and feet (recorded on Form 1, Annex 3.5).

For vaccination into the pectoral muscle, the penguin was held upright with one of the handler's hands controlling the head and the other supporting the bird's weight and holding the feet. The flippers were controlled by pinning them between the handler's arms and body.

For jugular blood sampling, the handler was seated with the penguin held between the handler's legs, facing away from the handler, with the flippers confined at the bird's sides. The head of the bird was gently but firmly held behind the eyes, with the right hand, and the neck fully extended and pulled laterally over the handler's right thigh. The handler covered the bird's eye with the left hand, to reduce stress.

The bleeder occluded the left jugular vein with their left thumb and inserted the needle into the raised jugular vein. After blood was collected into the syringe, the handler placed moderate pressure on the venepuncture site, and the handler then took over this task and returned the head to an upright

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position. Pressure on the vein was maintained and the bird was held still for at least 2 minutes after the needle was withdrawn, to prevent haemorrhage.

The right vein was only attempted if sampling was unsuccessful after two needle insertions into the vein on the left side. The right vein is larger but runs close to the oesophagus and is awkward to access for a right-handled sampler.

The penguin was placed in a pen with others already processed and monitored every 15 minutes for an hour for signs of allergic reaction to the vaccine and (or) for haemorrhage from the jugular vein.

The maximum handling time allowed was 5 minutes. A failed attempt was classified as failure to obtain the minimum volume (3ml) of blood before either 5 minutes had elapsed since the start of handling, three needle insertions, respiratory distress or bleeding considered excessive by the veterinarian.

A maximum of 5ml of blood was sampled from the jugular vein, using a 21 G, 1 1/2 inch hypodermic needle on a 5ml syringe. A minimum of 3ml of blood was required, to ensure enough serum for analysis. 3ml of blood was expected to yield approximately 2ml of serum (based on an assumed haematocrit of about 35%).

The University of Kentucky Division for Animal Resources provides guidelines on safe volumes of blood to be sampled from animals. Blood volume is estimated at 6% of body mass in chickens, so a 3.5kg penguin should have approximately 210ml of blood. The website states “If the amount of blood volume removed is… 10%, allow 2 weeks recovery”, so a maximum of 21ml should be sampled every two weeks in a 3.5kg penguin. Mazzaro *et al.* (2013) sampled 10ml of blood from African Penguins once a week for 5 weeks and found no adverse effects.

3.2.7. Monitoring and preparation for adverse effects

Any clinical signs or adverse effects were recorded (Form 3, Annex 3.5) and managed. Little to no reaction to the vaccine was expected, but the birds were monitored for an hour after vaccination and sampling so that any allergic reaction or injury could be detected and treated, according to the SOP in Annex 3.3, summarised below. The penguins’ behaviour was also monitored, to assess stress levels.

Any bird showing signs of respiratory distress or adverse reaction to the vaccine was to be placed in an oxygen box or oxygen was to be supplied via endotracheal tube under sedation, if necessary. Adrenaline (at 1ml/kg) and antihistamine would be administered and intravenous fluids if necessary. If bronchoconstriction was suspected due to difficulty breathing and wheezing, salbutamol could be administered via an asthma pump or nebulizer.
In the event of excessive bleeding, pressure will be maintained for a further five minutes and, if necessary, intravenous fluids will be given after inserting an intravenous catheter under general anaesthesia.

The birds were housed for approximately 2 hours in smaller temporary pens while procedures were performed, but were returned after each day’s sampling to the pen with other resident penguins.

For longer-term effects of the vaccine and blood sampling, abnormalities in behaviour, habitus and food consumption were noted daily at feeding on a monitoring sheet (Form 4, Annex 3.5), and the resident vet was asked to examine any bird that appeared abnormal.
Behaviour monitoring is detailed in the SOP in Annex 3.4.

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3.2.8. Sample handling

After drawing the blood, the needle was removed from the syringe and the blood was transferred gently to a 5ml gold-topped serum separator tube (SST). Sealed serum tubes were gently inverted 180° and back 5-6 times to expose the blood to the silica clot activator.

Tubes were kept at room temperature for at least two hours before centrifugation at 1690 to 2254 g for 15 minutes. The lower speed was used after haemolysis of samples was observed, but this was probably more due to the room temperature storage after sampling. Room temperature is generally advised for mammalian samples and anecdotally assists with clotting in ostrich samples, but an experienced vet (R. Vanstreels) advised cooling penguin samples immediately (SOP included in Annex 3.2).

Serum was refrigerated and kept cold during transport to the laboratory, usually two to three days after sampling. The Day 430 plasma sample was frozen at -20°C until testing.

Blood from the hub of the needle/ neck of the syringe was used to draw up blood in two microhaematocrit tubes. Blood from the microhaematocrit tube was used to dispense a drop of blood to make a blood smear.

Microhaematocrit tubes were plugged and stored upright until centrifugation later for 5 minutes at 14 490 g (KHT-430B Gemmy Industrial Corp. haematocrit centrifuge).
The microhaematocrit tube was used for the assessment of packed cell volume (PCV) and total serum protein (TSP), recorded on Form 2 (Annex 3.5). The blood smear, PCV and TSP were assessed, as is done routinely at SANCCOB, to monitor general health. PCV was used to monitor the effect of repeated sampling.

3.3. Pilot study

SANCCOB requested a pilot trial before agreeing to a full vaccine study, to assess potential side effects. The trial study was conducted using tobacco leaf extract, without the vaccine component. There was insufficient inactivated vaccine to include it in the pilot, but also evidence of safety in penguins from previous studies.

The pilot was performed on 15 and 30 July 2020. A 50:50 mixture of plant extract and the Montanide™ ISA 71 VG oil adjuvant was injected. A tobacco leaf extract was prepared at the Council for Scientific and Industrial Research (CSIR) in exactly the same way as the H5 VLP vaccine, though it was not diluted and therefore contained a higher concentration of tobacco plant leaf proteins than the PBS-diluted H5 VLP vaccine. The same batch of Montanide ISA 71 VG adjuvant was also used.

Two female penguins, recovering from pododermatitis, that excluded them from the main trial, were injected with 0.25ml of the mixture intramuscularly in the breast muscle. Both were injected on the first day and one on the second occasion (the other had started moulting).

3.4. Laboratory testing

3.4.1. Serology

Serological tests performed at the Western Cape Provincial Veterinary Laboratory (WCPVL) included an influenza A enzyme-linked immunosorbent assay (ELISA) (IDEXX Influenza A Virus Antibody Test Kit, part 99-53101, IDEXX Laboratories), performed according to the manufacturer's instructions, and haemagglutinin inhibition (HI) tests using standard test reagents from Deltamune (Pty) Ltd and the

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Southern African Development Community (SADC) protocol (Kangumba, 2011; Kangumba *et al.*, 2009), in line with the WOAH-recommended methods for non-chicken species. The HI antigens employed were:

- A/duck/South Africa/811/2004 (H5N1; LPAI)
- A/ostrich/South Africa/N227/2004 (H5N2; HPAI)
- A/chicken/South Africa/Villiers/2017 (H5N8; HPAI; 99.8 % identical to A/Speckled pigeon/South Africa/08-004B/2017 (H5N8))
- A/mute swan/England/AVP 18 001986/2017 (H5N6; HPAI; 99.1 % identical to A/chicken/Villiers/2017 (H5N8))

Additionally, each 0.5 ml serum was treated with 0.5 µl neuraminidase from Vibrio cholera (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) and incubated at 37°C overnight, followed by inactivation in a 56°C water bath for 30 minutes and adsorption with chicken red blood cells (RBCs), to avoid non-specific haemagglutination.

Samples taken on day 0 were also tested with Eurasian-lineage H5N1 and H5N2 HI antigens to exclude the presence of cross-reacting antibodies from any previous H5 AIV infection.

Excess serum was stored frozen at WCPVL until the end of the study and the serum was then transferred to the Poultry Research Laboratory at the Faculty of Veterinary Science, UP.

3.4.2. Blood smear assessment:

Blood smears were scored according to the numbers and types of leukocytes (heterophils (H), lymphocytes (L), eosinophils (E) and monocytes (M)), age of erythrocytes (regeneration, an indication of anaemia) and numbers of heterophils with toxic granulation per high power field (HF; 500 times magnification using a 50x oil immersion objective lens) by the SANCCOB veterinarian (Vanstreels and Parsons, 2017).

Toxic granulation indicates severe bacterial or fungal infection and erythrocyte regeneration refers to the percentage of erythrocytes that are young/blast cells. This rises after blood has been lost or excess red blood cells destroyed. Table 5.3 shows scores associated with different cell counts.

Table 5.3: Scores assigned to African Penguin blood smears based on immune cell counts, per high power field

Score	Leukocytes: cell count per hf				Toxic granulation of heterophils	Erythrocyte regeneration
	H	L	M	E		
Normal	0-4	0-2	0-1	**0-1**	0%	-
1+	4-8	2-4	1-3	**1-3**	1-20%	**1-15%**
2+	8-12	4-8	3-6	**3-6**	20-40%	**15-50%**
3+	**>12**	**>8**	**>6**	**>6**	>40%	**50-90%**
4+	-	-	-	-	-	**>90%**

3.4.3. PCV and TSP assessment:

A cut-off, based on the normal PCV of Home Pen penguins, was used to determine if a bird must have a PCV done before the next sampling event to ensure blood collection will not dangerously lower red blood cell levels.

Two historical PCV and TSP readings were extracted for each of 21 of the trial penguins. Frequency distributions did not appear normal so interquartile ranges (IQR) were calculated. The IQR for PCV was 40-47 and for TSP 6-7.4. Penguins with a previous PCV of 35 or less would be checked before sampling.

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3.5. Quality control, record-keeping and document storage

3.5.1. Quality control

Storage of vaccine: a digital max/min thermometer was kept with the vaccine from delivery until administration of the booster vaccine and max, min and current temperature were recorded daily at the same time on a data sheet.

Vaccines were dispatched, on ice, and with two frozen 500ml bottles of viral transport medium, in insulated boxes, by courier on 6 August 2020. They were delivered on 11 August (5 days later) at 16°C and then slowly cooled again, to approximately 4°C, and remained between 0.1 and 7.7°C until 10 October.

As required by SAHPRA, a monitor was appointed to ensure that the vaccine trial was carried out according to the proposed protocol. A State Veterinarian with relevant experience was requested to fulfil the role.

3.5.2. Record keeping

Treatment groups: the list of treatment group numbers and the assigned vaccine and dosing schedule were to be stored digitally and in hard copy by the SANCCOB vet and the monitor until data analysis was finalised, when copies would be transferred to the principal investigator.

A printed data collection/ check sheet was completed when birds were examined, vaccinated and sampled. A separate sheet was used to record the results of PCV, TSP and blood smear analysis. Once complete, these sheets were photographed on a mobile phone that automatically backed up photographs to the cloud. Photographs were downloaded and stored digitally on the investigator's PC and remained backed up in the cloud. The data were also transferred to an MS Excel spreadsheet, which was stored both on a PC and also backed up to the cloud.

Daily monitoring sheets were completed on paper by those feeding the birds. These were photographed weekly and sent to the investigator, who stored two copies (on the cloud and on a PC) and transferred results to a spreadsheet, also stored in two places.

Laboratory reports were saved both on a PC and backed up to the cloud. Results were transferred to a spreadsheet, also stored in two places. The spreadsheet recorded the laboratory reference numbers so that if any or all laboratory reports are lost, they can be requested from the laboratory.

All records were stored digitally by the investigator and, when the study is complete, digital and paper versions will be transferred to the University of Pretoria, in line with requirements for storage of raw data.

3.6. Permits

Section 20 approval under the Animal Diseases Act (Department of Agriculture, Land Reform and Rural Development, DALRRD): 12/11/1/7 (REC256-19): valid until 30 April 2022.

South African Health Products Regulation Authority (SAHPRA): VCT/08/2020 permission to use an unregistered veterinary medicine and to perform a clinical trial.

Research Ethics and Animal Ethics approval from the Faculty of Veterinary Science: REC256-19 valid until 7 July 2022 (extended).

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3.7. Data analysis

3.7.1. Serological results

When antibody titres are log-transformed by calculating the log to base 2 of the reciprocal of the dilution (e.g. a titre of 1:16 is transformed to a $\log_2$ titre of 4), the frequency distribution of titres can then be assumed to be lognormal (Thrusfield, 2007). Therefore, tests that assume normality can be used to compare groups, or titres within a treatment group, for different sampling dates. However, zero titres cannot be used to calculate means because the logarithm of zero is 'minus infinity', which cannot be included in a calculation.

For each time point, the proportion of the group that achieved an HI titre of 32 or more (the titre expected to protect against mortality; WOAH, 2021), based on the H5N8 HI tests, the geometric mean antibody titre (GMT) and $\log_2$ titre standard deviation (SD) were reported for each group and the $\log_2$ means were compared statistically between groups (different vaccines and dosing schedules). The geometric mean titre (GMT) is the antilog$_2$ of the arithmetic mean (or $2^{a.\ mean}$) of the $\log_2$-transformed titres. The number of non-zero titres used to calculate GMT was also reported.

A pooled (Student's) t-test, assuming equal variances, was used for comparisons of means of log-transformed titres between two groups. A two-tailed P-value of <0.05 was considered significant. The assumption of equal variances was checked using an F-test, at a 1% significance level. The 95% confidence interval (CI) of the difference in means between two groups was calculated in EpiTools.

3.7.2. Health monitoring

Daily observations at feeding were transcribed into MS Excel worksheets with a column for each day and a row per bird. Abnormal behaviour was recorded as yes/ no and summarised as counts, weekly. Feeds Missed were recorded as 0, 1, 2 and a percentage of total feeds was calculated weekly (from a total of 14 feeding events). Trends were also monitored.
Body mass gain or loss was calculated from the previous mass recorded and assessed as a percentage of body mass on day 0. Losses of more than 5% were flagged.

4. Results

4.1. Pilot study

No adverse effects were observed on the day of injection of the tobacco leaf extract, except possible irritation at the injection site in one bird, which was observed preening the site. She also appeared slightly depressed in the 20 minutes after the injection but was otherwise normal. The other penguin had an elevated respiratory rate (12 breaths per minute) 10 minutes before and after the injection but this decreased to between 6 and 8 breaths per minute for the rest of the observation period, and behaviour and physical characteristic did not change. The latter bird received the second dose and had a slightly elevated respiratory rate (10 to 12 breaths per minute) in the 40 minutes after the injection, and appeared slightly nervous and stressed, but otherwise appeared normal. She was alone, which is expected to have contributed to stress.

The rehabilitation staff reported normal appetite on the days of injection and did not report any unusual behaviour at feeding on subsequent days.
Both birds underwent a health check and clinical examination on 23 July, eight days after the first injection, and appeared normal, with no swelling at the injection site and no abnormalities on their blood smears, except for mild inflammation for one bird. Both birds were checked again on 5 August, six days after the second injection, and again no swelling was palpated in the breast muscle. The blood

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smears both showed mild inflammation, with a slight increase in macrophage count in the bird that received a second dose of plant extract. This may, however, also be attributed to chronic pododermatitis, for which both birds were still receiving treatment at the time.

4.2. Sampling events and sample sizes

The trial started on the 15th of August 2020, with the administration of the first vaccine dose and Day 0 blood sampling. The last sampling of Group 2 (Inactivated vaccine) was on the 29th of May 2021 (day 287) and the last Group 1 (VLP vaccine) sample was taken on 19 October 2021 (Day 430). Sample sizes for each sampling event are shown in Table 5.4.

The group vaccinated with the H5 tobacco-produced virus-like particle (VLP) vaccine (Group 1) developed bacterial infections due to unforeseen contamination of the VLP vaccine, which led to one bird being excluded on day 14, due to illness and a swelling in the breast muscle, and seven birds on day 28, due to illness (Table 5.4). All in the group were withdrawn from the trial after day 28, though all recovered fully with treatment. One bird from Group 1 was sampled on day 430.

On days 56, 84 and 112, two, three and two moulting birds were excluded respectively from the sampled inactivated vaccine group (Group 2), and no blood could be obtained from one bird on day 175. The unboosted inactivated vaccine group was excluded after day 224, when $\log_2$ HI titres for all birds in that group were less than 16 for the second time.

A last sample was tested opportunistically in October 2021 (Day 430). Blood was drawn from a penguin that had been vaccinated with the plant-produced virus-like particle vaccine, for transfusing an anaemic bird admitted for rehabilitation. The blood had been mixed 10:1 with sodium citrate but was subsequently observed to contain small clots. It was unsuitable for transfusion but SANCCOB approved testing of the plasma as part of the vaccine trial.

4.3. Serology

All birds were avian influenza seronegative on day 0 and this sample acted as the negative control for postvaccination samples.

4.3.1. ELISA results

All ELISA tests performed on Group 1 were negative. In Group 2, there was general agreement between ELISA and HI tests (positive/ negative; 82/105 tests), though only 64% of HI-positive (≥16) samples were ELISA-positive.

4.3.2. H5N6 vs H5N8 haemagglutination inhibition tests

Of 111 samples tested, 107 H5N6 tests (96%) had titres within one order of magnitude of the H5N8 titre; 60 were equal to the H5N8 titre, 45 were lower and two were higher than the H5N8 titre.
For three samples, the H5N6 titre was two orders of magnitude lower than the H5N8 and for one it was four orders of magnitude lower.

The H5N8 titre was analysed alone for the remainder of the analyses, given the similarity to the H5N6 titre.

4.3.3. Mean H5N8 haemagglutination inhibition titres

The birds that received the inactivated vaccine (Group 2) had a H5N8 antibody geometric mean titre (GMT) of 40 ($\log_2$ SD= 2.1, n=12) at day 14 after vaccination compared to a GMT of 424 in the VLP-

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vaccinated group (Group 1; P=0.0008, n=11; difference 95% CI=1.6-5.2), and a GMT of 85 (SD= 1.3) at day 28 (n=12) compared to a GMT of 446 in Group 1 (SD= 2.9; n=5; P=0.0319, difference 95% CI=0.3-4.5; Figure 5.1, Table 5.4).

Table 5.4: African Penguins vaccinated with an H5 avian influenza virus-like particle (VLP) vaccine (Group 1) and one (Group 2a) or two (Group 2b) doses of an inactivated high pathogenicity avian influenza H5N8 vaccine: Sample sizes (n), proportion of group that seroconverted with protective antibody titres (>32; PP), if applicable, and mean log_2 H5 influenza subtype-specific antibody titre and SD.

	Days after primary vaccination										
	0	14	28	56	70	84	112	175	224	287	430
n (n titres >0)											
Group 1	12	11	5	0	0	0	0	0	0	0	1
Group 2a	5	6	6	4	6	5	5	5 (4)	6 (2)	0	0
Group 2b	6	6	6	6	6	4	5	6	6	6 (5)	0
PP (%)											
Group 1	0	91	80	-	-	-	-	-	-	-	0
Group 2a	0	33	100	25	33	0	0	0	0	-	-
Group 2b	0	83	100	67	100	100	80	17	17	0	-
Mean (SD) log_2 titre											
Group 1	-	8.7 (2.1)	8.8 (2.9)	-	-	-	-	-	-	-	4.0
Group 2a	-	4.3 (2.1)	6.2 (1.0)	4.0 (0.8)	4.2 (0.8)	3.2 (1.1)	2.0 (1.2)	1.5 (0.8)	2.0 (1.0)	-	-
Group 2b	-	6.3 (1.8)	6.7 (1.6)	4.3 (1.5)	8.2 (1.6)	7.5 (1.3)	5.4 (1.1)	3.5 (1.5)	3.2 (2.2)	2.2 (1.2)	-

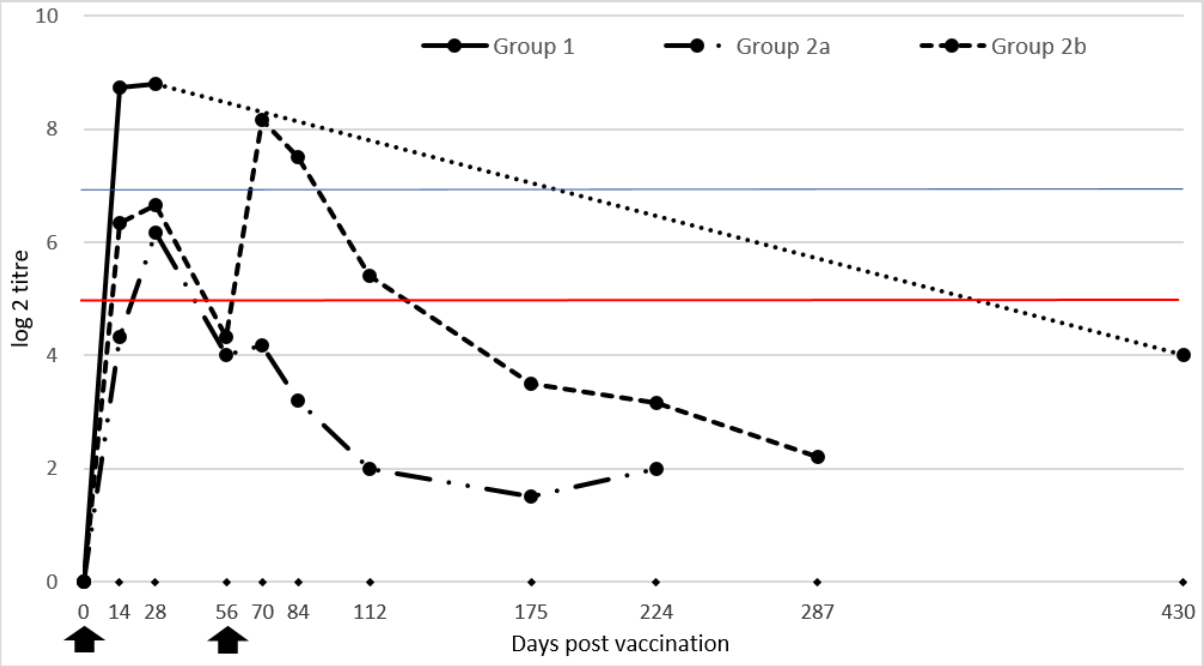


Figure 5.1: H5 influenza subtype-specific antibody response in African Penguins vaccinated with two different H5 vaccines.
Group 1 received an H5 avian influenza virus-like particle (VLP) vaccine and Groups 2a and 2b received one and two doses, respectively, of an inactivated high pathogenicity avian influenza H5N8 vaccine. The days on which the vaccine was administered are indicated by arrows. The red and blue lines indicate the minimum antibody titres expected to provide protection against infection and reduction in virus shedding respectively (WOAH, 2021).

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Although there is an apparent difference in GMT between Groups 2a and 2b on Days 14, 28 and 56, the biggest difference (Day 14) was not statistically significant (P=0.1).

Seven of twelve (58%) in the Group 2 had titres that should protect against disease and mortality (>32; WOAH, 2021) on day 14, compared to 10/11 (90%) in the VLP group. All birds in Group 2 had titres >32 by day 28 and Group 1 was still at 90%.

Four of twelve (25%) in Group 2 had titres that should reduce challenge virus replication and shedding (> 128; WOAH, 2021) on day 14 and day 28, compared to 10/11 (91%) and 4/5 (90%) in the VLP group, respectively.

All titres in Group 2 had dropped lower than 128 by Day 56 and the GMT in Group 2a had dropped below 16 (the titre considered positive) by day 84.

In Group 2b, the GMT rose to 287 on day 70, with 5/6 birds >1:128), compared to a GMT of 18 in Group 2a (P=0.0002, difference 95% CI=2.4-5.6) and had dropped below 16 by day 175 (GMT = 11). However, two birds maintained titres >16 until Day 224 and one bird's titre was still >128, the bird that had the highest HI titres from day 14 to 56. All birds had titres <16 by day 287.

The bird vaccinated with the VLP vaccine and sampled on day 430 had a H5N8 titre of 16.

4.4. Adverse effects

4.4.1. Adverse effects observed

One of the penguins was reported as sick on 27 August (day 12). He was anorexic and dehydrated and the blood smear indicated severe inflammation. At day 14 (29 August) a total of four of twelve penguins that received the plant-based vaccine had swellings at the injection site and their blood smears showed severe inflammation (>13 heterophils per high power field (HF), toxic granulation and >2 lymphocytes/HF). A fifth bird had a similar blood smear but no noticeable swelling. Four of these five had lost between 12 and 17% of their body weight in the previous two weeks, and the feeding records showed they had missed a large proportion of feeds. The feeding sheets had not been monitored by a single person and different staff were feeding from one feed to the next and were not noticing accumulated missed feeds over time. Abnormal behaviour had been recorded in 30/156 penguin-days for the VLP group, compared to eight in the inactivated vaccine group.

About half of the inactivated vaccine group had also lost some weight but a maximum of 8%, except one who had lost moulting weight. None of these had any signs of inflammation on their blood smears and they had generally been eating better than the other group.

On day 28 (12 September), seven birds in the VLP group were excluded from sampling because they did not fulfil the health criteria for sampling. The health criteria were set as: normal appetite and behaviour, stable or increasing weight, PCV above 35 that is stable or increasing and a heterophil count below 13 cells per high power field (<13/hf).
The situation was re-assessed on 6 October 2020. In the previous two weeks (since 21 September), the behaviour and appetite of the birds had returned to normal in Home Pen. Only 11 (8 in the VLP group) instances of unusual behaviour (out of 308 penguin days) were recorded in two weeks compared with 23 counts (19 in the VLP group) in the two weeks before (7-20 September). Two birds were being monitored more closely in SANCCOB's intensive care unit (ICU).

In the inactivated vaccine group, all of the birds fulfilled the health criteria to qualify for another sampling. Only one bird was mildly concerning and had unusual behaviour noted on two of the

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previous 14 days. No abnormalities were detected on a clinical examination and only mildly increased heterophil numbers were observed on a blood smear.

In the VLP group, the weights of almost all the birds decreased after the vaccination, but were improving. All but three had regained the weight that they lost. Two had not been weighed recently but were otherwise healthy.

Heterophil counts had returned to <13/HF in all but two birds. These two birds' blood smears had improved markedly by 10 October, however, and all heterophil counts were <13 by 24 October.

PCVs mostly decreased after the vaccination but were >35 in 6 birds and were increasing or stable in 4 of these birds. One bird's PCV decreased but was unusually high at the previous check, so was not concerning. The bird that was not regaining weight had a decreasing PCV and a first abnormal blood smear on 10 October. He required surgical debridement of a thoracic mass on 13 October. Ten birds eventually developed thoracic muscle masses.

PCVs were below 35 in 6 birds, but all except one were on the rise or stable. All were >38 by 24 October. The exception had been the only sick bird recently (with continued high heterophil counts) and had been undergoing a second round of treatment. On 2 October, surgery had been done to debride the swelling in the breast muscle, which was found to be an abscess. Sutures were removed on 27 October and no further abnormalities were recorded.

4.4.2. Treatment of affected penguins

The five affected birds identified on day 14 were placed on a course of antibiotics (clindamycin) and anti-inflammatories (meloxicam) and the SANCCOB vet observed them closely to see if any other treatment was necessary. Another two birds were treated in the next two days because weight loss and deteriorating blood smears were noted.

The rehabilitation staff were requested to send copies of the feeding sheets to the primary investigator every day. The results were entered into a spreadsheet to monitor the birds over time and detect any trends in missed feeds and abnormal behaviour as early as possible. The SANCCOB vet was advised of any negative trends.

From 4 September, eleven birds received antibiotic treatment with florfenicol. It was the only antibiotic to which both of the bacteria identified in the remaining vaccine were susceptible on antibiogram.

Most treatment courses were completed by 16 September but one bird received a second course in late September/ early October, as well as surgical debridement of an abscess. One bird did not require treatment and only showed a mildly abnormal blood smear.
Eventually, two birds required surgical debridement of the thoracic abscesses: on 2 and 13 October respectively.

4.4.3. Investigation of VLP Vaccine Contamination

Bacteria *(Pantoea (Enterobacter) agglomerans* and *Enterococcus spp*.; Vet Diagnostix report 20-18620) were cultured from the combined vaccine and adjuvant, mixed in the same Eppendorf tube (stored closed and refrigerated) as the vaccine administered to the penguins. These bacteria are common in the environment. Additionally, although there was no bacterial growth from a sample of the adjuvant (Montanide ISA71-VG), *Escherichia vulneris* was cultured from the frozen stock solution of the vaccine, already diluted in PBS (UP Dept. Veterinary Tropical Diseases bacteriology report B778-20). The Vet Diagnostix bacteriologist advised that *E vulneris* could have been present in the sample

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presented to Vet Diagnostix because the colonies look similar to *Pantoea* and the biochemical tests used for identification are similar, so it could have been missed. The absence of *Enterococcus* in the stock solution could indicate a second contamination event, however.

The affected birds were successfully treated with clindamycin and florfenicol, chosen based on the antibiogram and anti-inflammatory drugs and, in two cases, surgical debridement of abscesses. Material from one abscess was submitted for bacterial culture and *Corynebacterium amycolatum* (a common skin resident), a *Streptomyces* and a *Streptococcus* from the Lancefield Group D were identified (Vet Diagnostix report 20-19689).

5. Discussion

The aims of this clade 2.3.4.4b H5N8 high pathogenicity avian influenza vaccine trial in African Penguins were to assess and compare the magnitude and duration of antibody response induced by two vaccines, each administered according to two different schedules, and to assess the safety of the vaccines in penguins.

5.1. VLP vaccine contamination

Vaccine safety was confirmed for the whole virus inactivated vaccine administered to Group 2 but unfortunately not for the tobacco leaf-produced virus-like particle vaccine administered to Group 1. The safety of the VLP vaccine in penguins cannot be confirmed with the data available, though the cause of illness in Group 1 is believed to be due to environmental bacterial contamination of the vaccine and reactions to the vaccine itself seem unlikely. Tobacco-produced VLP influenza vaccines in humans produced mild, self-limiting reactions at worst (Cummings *et al.*, 2014; Landry *et al.*, 2010; Pillet *et al.*, 2019; Ward *et al.*, 2014). Additionally, the pilot study conducted in two penguins prior to the trial produced no reactions, despite the administration of undiluted plant proteins. The process of administering the vaccine as a cause of contamination can also be ruled out as the other treatment group was entirely without adverse effects, the two vaccines were administered blind and bacteria were cultured from the VLP stock solution.

The suspected contamination occurred possibly via the PBS diluent, the sucrose density gradient purification or, more unlikely, the trehalose stabilizing agent, supplied as a powder. Even if the trehalose was not a source of contamination, it was likely a convenient nutrient source to support the growth of the low-level bacterial contaminant in the VLP vaccine stock, and the break in the cold chain (delayed delivery of the vaccine in Cape Town) may have exacerbated the situation. *Pantoea (Enterobacter) agglomerans* strains can be pathogenic to both plants and animals (Cruz *et al.*, 2007) and their association with plants should not be ignored here, though its significance is unknown. It underlines the importance of sterility testing plant-produced vaccines and ensuring the health of the plants, even with aseptic technique during vaccine production.

The addition of antibiotics to the vaccine was considered, to eliminate bacterial contaminants before administering the second dose, but production of endotoxin from the gram-negative bacteria, *Pantoea and Escherichia spp*, was a serious concern. The vaccine could have been filtered, but further handling of the Group 1 penguins, for vaccination or sampling, was not permitted by SANCCOB anyway. Although all the penguins recovered from the contaminated VLP vaccine, SANCCOB did not permit any further sampling or vaccination after day 28. All effort was made by the researcher to provide evidence that the birds had recovered health and normal behaviour, to justify continuing with sampling at least, but SANCCOB felt that the birds should not suffer any further fear or discomfort because of the trial.

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The WOAH Terrestrial Manual provides guidelines for establishing protocols for the aseptic preparation of vaccines that should prevent future similar regrettable situations (WOAH, 2021). The addition of antibiotics to vaccines is mentioned, but they add to the risk of vaccine reactions and are discouraged by some, who prefer prevention of contamination, via aseptic techniques. All VLP future vaccine batches, produced by those involved in this project will be sterility tested prior to use. Abolnik *et al.* (2022b, 2022a) have been fulfilling this commitment by performing aerobic and anaerobic cultures of their H5 and H6 VLP vaccines before administration.

The illness in the VLP group would have been detected sooner, if there had been better monitoring of the feeding records. The primary investigator should have requested daily feedback from the beginning to be able to have an overview of the penguins' behavioural trends. When different people are feeding and monitoring from day to day, and are not reviewing previous records, problems can be missed.

Regarding the break in the cold chain, the presence of the 5% trehalose stabiliser in the VLP vaccine, the presumed slow rise in temperature during transit, and the results of a literature review provided reassurance that the vaccines should not have been damaged significantly, though the bacterial contamination was unforeseen. Donaldson *et al.* (2018) found that enterovirus VLP produced by baculoviruses in insect cells remained stable for 1 month at 25 and 37°C in sodium phosphate buffer and they referenced a study that showed increased stability of VLP with the addition of trehalose. Chu *et al.* (2016) found that liquid whole inactivated influenza H1N1 virus vaccine, stored at 25°C for one month stimulated antibodies with similar HI titres to the positive control (vaccine stored for one day at 4°C).

5.2. Serological response to vaccination

A single dose of the inactivated clade 2.3.4.4.b H5N8 HPAI vaccine induced protective antibody levels by day 14 after vaccination but levels had dropped by day 56 (8 weeks). Levels were never high enough to be considered able to reduce virus replication and shedding.

Booster vaccinations in other studies were administered at five or six weeks after the primary vaccination (Bertelsen *et al.*, 2007; Furger *et al.*, 2008; Lécu *et al.*, 2009; Philippa *et al.*, 2007, 2005). Though administered at least two weeks later after the initial vaccination than in the other studies, the booster in this study, administered at eight weeks, induced similar antibody levels to those described in penguins previously. The booster had induced antibody levels high enough to suppress shedding by day 84, although they had dropped below suppressive levels by day 112. Furger *et al.* (2008) found a GMT of 9.7 in ten Humboldt (*Spheniscus humboldti*) penguins at 26 weeks (182 days) after the initial vaccination and 21 weeks (147 days) after the booster. At a similar period after the booster (day 224) in this study, the GMT was nine, showing a similar rate of antibody decline. Earlier administration of the second dose, on day 28, should have prevented the Day 56 trough in this study, but probably would have shortened the period of protection.

One bird that received the booster showed H5N8 antibody HI titres >128 until Day 224. This cannot be seen as indicative of the vaccine's efficacy, given the normal variation in vaccine response between individuals (Zimmermann and Curtis, 2019). However, it is interesting to note that the bird in question was the primary "ambassador bird" for the rehabilitation centre, accustomed to visiting schools and regularly interacting with humans. It is therefore possible that the strong and long-lasting immune response in this bird was due to lower stress levels during handling for the study and maybe in the longer term. Chronic, rather than acute, psychological stress has been more strongly linked to poor vaccine response in humans (Van Loveren *et al.*, 2001; Zimmermann and Curtis, 2019) but the

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potential negative role of psychological, nutritional, heat and cold stress on immune response should be considered when planning any vaccination program (Cheville, 1979).

This study did not fulfil its aims, with only one dose administered and limited sample sizes and sampling points in Group 1, that received the VLP vaccine. However, it was possible to demonstrate that the penguins that received the virus-like particle vaccine had significantly higher antibody levels than the inactivated vaccine group on days 14 and 28. Additionally, the levels were high enough to suppress virus shedding. Group 2b, and penguins in other studies involving inactivated vaccines, only reached similar titres after the second dose. The persistence of antibodies to day 430 in one bird, albeit below protective levels, is also encouraging. Antibody titres decreased to the same level by day 56 in the unboosted inactivated vaccine group and by day 175 in the boosted group, so the titres due to the VLP vaccine, in this bird, lasted at least twice as long as in the boosted inactivated vaccine group. The bird sampled on day 430 was one of the three with the highest titres on day 14 and it is therefore possible that the GMT would have been lower than 16 on day 430, if more birds had been sampled. Birds in Group 2 that initially responded to the inactivated vaccine with high titres generally remained among the birds with higher titres and maintained antibodies longer.

The bacterial infections from the VLP vaccine may also have had a suppressive effect on antibody production, so it is possible that a sterile vaccine would perform better. Bacteria that invade immune cells, like some *Enterococcus spp* (da Silva *et al.*, 2022) may reduce vaccine immunogenicity, but there is little evidence that concurrent bacterial infections will have an effect, in general (Van Loveren *et al.*, 2001; Zimmermann and Curtis, 2019). The effect of the antibiotic treatment on antibody reactions to the VLP vaccine should also be considered. Clarithromycin, a macrolide similar to clindamycin, was found to suppress antibody responses to some human vaccines, in mice (Woo *et al.*, 1999). However, the effect of administering the antibiotic more than two weeks after the vaccine may not be significant.

The fact that influenza-specific HI antibody titres in plasma may also be lower than in serum (Defang *et al.*, 2012), should also be a consideration regarding the day 430 sample, but generally there is good agreement and correlation between matched serum and plasma samples (Morrison *et al.*, 2018).

All ELISAs performed on Group 1 were negative, illustrating the H5 VLP vaccine's DIVA capability. The Influenza A ELISA can still be used as the primary tool for serological surveillance, and the presence of vaccine-induced antibodies can be confirmed with HI testing. This is not possible with the inactivated vaccine. The ELISA detects antibodies to nucleoprotein, present in whole virus vaccines. DIVA is not yet vital in wild birds, where surveillance is currently aimed at detecting virus. However, serology could be valuable in studying subclinical circulation of AIV, especially if vaccination is employed.

It was not possible to include a control group as there were not enough birds available. However, all birds included were seronegative on Day 0 and no HPAI viruses were detected in the Western Cape province between June 2019 and 4 May 2021. The positive HI titre from Group 1 in October 2021 is therefore the only one that should be scrutinised. Hartlaub's Gull chicks were infected at SANCCOB in June 2021, probably because of an infected Kelp Gull kept in the same room while being treated for botulism. It died and tested positive for the HPAI virus. Two other Kelp Gulls in the same room tested positive but it was not possible to tell whether they were infected in the centre or before admission. Others in the room tested negative and no clinical signs were observed in the rest of the centre. It is believed that the outbreak was confined to that room and that there is a low chance that it caused infections in the penguins in the Home Pen. The H5 HI-positive penguin was ELISA-negative, which also provides some reassurance that natural infection did not take place. However the ELISA test appears less sensitive at similar HI-titres in the inactivated vaccine group, so this is not conclusive.

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Ideally, a vaccine is also tested by infecting animals with the pathogen in question and comparing the resulting clinical signs and quantity of virus shed in vaccinated and unvaccinated groups (Abolnik *et al.*, 2022a). However, given the endangered status of penguins, a challenge trial with live HPAI virus was not possible, especially at this early stage. Further assays, such as virus microneutralization tests, might be able to confirm adequate levels of antibodies that inhibit virus entry and block replication (Reber and Katz, 2013), but these are not currently available in South Africa.

6. Conclusions

Plant-based virus-like particles offer a promising alternative to conventional inactivated whole-virus high pathogenicity avian influenza vaccines. Though the results from this study were not able to address the objectives, the antibody levels induced by a single dose of the tobacco-leaf clade 2.3.4.4 H5 vaccine by day 28 after vaccination was at least equivalent to that of two doses of inactivated whole-virus vaccine by day 70. Additionally, while two doses of the inactivated vaccine provided protective antibody levels until about week 18, and one dose until about week 6, there is an indication that the VLP vaccine could provide protective levels for nearly a year. A vaccine that only requires a single dose and annual vaccination is a more realistic option than one that requires two doses and provides much shorter-term protection. A vaccine that can be administered by spray would be more practical, as individual handling and injection of each bird would be labour-intensive, require more carefully-trained personnel and probably be more disruptive to a breeding colony. However, should these disadvantages outweigh the benefits of vaccination, in a situation where smaller numbers of birds are at high risk, individual injection may become necessary.

The duration of VLP vaccine-induced antibody persistence and the epidemiology of the disease need further investigation, and the feasibility of vaccinating wild penguins needs in-depth assessment.
Implementation of a vaccination program should be preceded by the identification of clear objectives and gathering of as much information as possible on the available vaccines, the disease's epidemiology and the population structure and characteristics of the susceptible species. The vaccines' capabilities and disease epidemiology cannot be considered in isolation from each other, and will dictate the actions that need to be taken. The next stage is consideration of whether the actions required are possible with the resources available and whether their benefits outweigh potential consequences for the vaccinated population and ecosystem at large.

Chapters 3 and 4 of this dissertation described high HPAI-induced mortality in adult penguins compared to chicks and more severe outbreaks at colonies with high nest densities and with neighbouring colonies of highly susceptible species. The arrival of a new HPAI virus is to be expected, though prediction of the date is difficult. To prevent further major losses, these colonies high-risk colonies should be prioritised for vaccination. Vaccination with vaccines shown to provide lasting protection in poultry will likely be effective, though further trials in penguins would be ideal. However, though vaccination is the only reliable method to mitigate the effects of an HPAI outbreak, the process to get approval to vaccinate wild African Penguins is expected to be long and difficult.

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8. Annex List

Annex 3.1- SOP: Blinding

Annex 3.2- SOP: Clinical examination, blood sampling, vaccination and record-keeping

Annex 3.3- SOP: Treatment of adverse reactions to vaccination or blood sampling

Annex 3.4- SOP: Welfare monitoring and penguin handling procedures

Annex 3.5- Data collection sheets

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Chapter 6

Planning for disease outbreaks and mass mortality events in South African seabirds

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1. Introduction

Coastal seabirds in South Africa, including four endangered species, have been severely affected by high pathogenicity avian influenza (HPAI), both in 2018 and from 2021 to 2022. The conservation status of the species affected and the lack of preparedness that was demonstrated, particularly in 2018, has highlighted the need for contingency planning, training and readiness, at a national, provincial and local level.

Disease is mentioned as a risk for seabirds and water birds in various international population or ecosystem management documents, and HPAI is often listed as a threat following the original Goose Guangdong H5 lineage outbreaks in the 1990s and 2000s (Cromie *et al.*, 2012; Parsons and Vanstreels, 2016; Peterson and Ferro, 2016). However, it appears to have been largely forgotten after that and the recommendations to develop specific contingency plans were never followed, as large wild bird outbreaks did not occur again until 2018 in South Africa and 2021 in the northern hemisphere. There were very few practical guidelines available to assist with decisions in South Africa in 2018 and widespread frustration with the number of unknown epidemiological factors. There was also a high level of uncertainty about who should lead the response and where responsibility for different tasks lay.

Management plans and guidelines are far more widespread in 2024 (e.g. Government of South Georgia & the South Sandwich Islands, 2023; Scottish Government, 2023), and experience with outbreaks has provided some insight into options for response. However, a persistent lack of factual information has resulted in many guidelines taking a cautious and conservative approach and the options are still very limited.

Despite the lack of concrete information on proven best practice, outbreak preparedness plans and guidelines are still encouraged (Kleyheeg *et al.*, 2020; Ramey and Handel, 2022). HPAI outbreaks should be responded to quickly and efficiently and no time should be wasted in assigning responsibility and deciding on a suitable response. The aim of a preparedness plan should generally be to delegate specific roles beforehand and save time searching for information. There is unlikely to be a set of people standing by to deal with a seabird disease outbreak, so a team needs to be formed, probably by people who seldom work together or perform the required tasks, in order to respond. It should also help them to understand the problem and required actions or provide the information to help make decisions about actions.

The South African Guideline on “Development and Structure of a Disaster Management Plan” (Directorate: Policy Development and Regulatory Frameworks, 2017) requires identification of the “what”, “why”, “who”, “how”, “where” and “when”. A plan must describe what has been done already and what is still to be done, and with what tools, why it must be done, who should do it and how, where and when. Animal disease preparedness plans should identify different stakeholders and define their roles and responsibilities, describe communication channels and early warning systems, provide information on the disease(s), and lay out the actions to be taken in response to an outbreak.

The Food and Agriculture Organisation of the United Nation’s (FAO) document on “Good emergency management practice” (FAO, 2011) outlines the “cycle of good emergency management planning and disease control”, which moves from prevention of a disease to detection, response, recovery and back to prevention, with preparation remaining central. It is advised that these steps be described in four documents: 1) a preparedness plan, 2) contingency plans that describe the response, 3) operations manuals or standard operating procedures (SOPs) that detail specific tasks and 4) recovery plans that describe the process to return to normal activities.

A plan for future South African seabird HPAI outbreaks was requested at the final stakeholder meeting after the HPAI (H5N1) outbreak, in early 2022. It had already been identified as a fitting way to tie

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together conclusions from the other projects described in this dissertation, so L Roberts volunteered to lead the project. It was proposed that the plan should apply to any mass mortality event or disease outbreak in coastal birds, given the common stakeholders and general approach to most of these events.

The input of all stakeholder groups was sought, in an attempt to ensure that the plan would be helpful and feasible when implemented. Lessons learnt from responding to the two major South African outbreaks were incorporated, as well as observations about apparent disease risk factors.

This chapter describes the process to draft a national disease preparedness plan for seabirds, as well as a protocol specifically for HPAI and a HPAI contingency plan suited to the sub-Antarctic Marion Island, which includes information on HPAI in marine mammals. It assesses the success of the exercise and makes recommendations for similar activities and for further actions needed.

2. Objectives

1. To draft a South African seabird disease outbreak preparedness plan, with the cooperation of all main stakeholders, that will:
    a. provide higher level decision-makers with an overview of what is required to prepare for and respond to a disease outbreak in coastal seabirds and
    b. guide site managers in writing their own specific contingency plans
2. To assess the process used to develop the preparedness plan, via questionnaires administered to stakeholders before and after a workshop
3. To draft a disease-specific protocol for HPAI in South African seabirds
4. To draft a site-specific HPAI contingency plan for Marion Island

3. Methods

3.1. Process to draft the South African seabird disease outbreak preparedness plan

A meeting was held at the Western Cape Provincial Disaster management Centre (WC PDMC) in February 2022 for role-players to give final feedback on the response to the 2021 mass mortality of seabirds, mainly affecting Cape Cormorants. Deaths had dropped to normal levels and most response operations had ceased. It was concluded that a workshop should be held to plan for future responses.

WC PDMC officials met with representatives from Western Cape Veterinary Services, CapeNature and Department of Forestry, Fisheries and Environment (DFFE), Directorate: Oceans Conservation Strategies in March to decide on the goal of the workshop. It would be aimed at drafting a contingency plan, and would be led by Western Cape Veterinary Services (L Roberts) and DFFE. The results would feed into the research for this dissertation.

L Roberts drafted the plan's framework, structured according to the South African National Disaster Management Centre of South Africa's "Development and Structure of a Disaster Management Plan", published in the Government Gazette in May 2017 (No. 40865):

Chapter 1 – Introduction and background

Chapter 2 – Constitutional, legislative and policy mandates

Chapter 3 – Integrated Institutional Capacity

Chapter 4 – Risk Assessment

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Chapter 5 – Disaster Risk Reduction

Chapter 6 – Preparedness planning Chapter 7 – Response

Chapter 8 – Recovery

The sections were partially populated before the workshop. Some were provided with bullet points or sub-headings, that needed discussion in the workshop, and others had suggested content, based on experience during the preceding outbreaks and on documents drafted during outbreaks. A list of disease threats for the Risk Assessment section was obtained from Parsons and Vanstreels' "Southern African Seabird Colony Disease Risk Assessment – December 2016".

Approximately one hundred people were invited to the workshop, including all major stakeholders in the Western Cape and key role players in the other coastal provinces (Northern Cape, Eastern Cape, and KwaZulu-Natal):

- provincial and district municipality disaster centres as well as the national disaster centre,
- other Western Cape provincial and municipal officials, mostly related to Environmental Affairs and including Veterinary Services,
- the national Department of Environment, Forestry and Fisheries (DFFE),
- managing conservation authorities, i.e. CapeNature and SANParks
- rehabilitation centres and aquaria that perform rehabilitation, including aquaria in the Eastern Cape and KwaZulu-Natal,
- the Society for the Prevention of Cruelty to Animals (SPCA), and
- the South African Police Services

At the workshop, after an overview of lessons learned from the previous HPAI outbreak, the day was spent going through the draft plan and discussing what needed to be included, adding some information and some of the logistical aspects.

After the meeting, inputs were incorporated and some text added where information was available (role of veterinary services, lists of Threatened and Protected Marine Species (TOPMS)). It was emailed out a month later, different sections were allocated to specific individuals and their organisations, and a deadline for input of two weeks was set.

It was necessary to send further requests for input and to organise a follow up meeting with SANParks and CapeNature in November 2022. Here, the content requirements were discussed further.

The approach was then changed, from requesting all role-players to add content, to drafting as complete a document as possible and requesting correction and answers to specific questions. The more complete version was circulated to a much smaller group of key role-players in March 2023.

The input received on this draft was included in a final version.

3.2. Questionnaires to assess the preparedness planning workshop

To measure the value of the workshop and its structure, one survey was administered beforehand and another afterwards, to measure expectations and needs and whether these were met.

Two days before the workshop, the attendees were emailed a hyperlink to the first anonymous online questionnaire, created and administered in Google Forms. The questions were:

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1. Have you dealt with a coastal seabird disease outbreak/ mortality event? (Yes/No) [If the respondent chose no, the survey ended]
2. If yes to (1), what role did you play in the event?
    a. Coordination/ Managerial
    b. Operational (on the ground)
    c. Supporting/ advisory
    d. Administrative/ Regulatory
    e. Monitoring/ reporting
3. a. Were you and your organisation prepared for the event in any way (you knew what to do and could do it)?
   b. If you were prepared, please provide some details
4. From what three aspects do you feel you and your organisation were most unprepared?
5. What were the three biggest challenges you faced during the event (whether you were prepared or not)?
6. Would prior planning have helped with any of the above challenges?
    a. Yes to all
    b. No to all
    c. Other
7. What are the three most important aspects you want covered in a contingency plan?
    a. Same as answers to question 4
    b. Same as answers to question 5
    c. Same as answers to questions 4 and 5
    d. Other

After the workshop, they received another link to a second questionnaire to gauge whether the workshop was perceived as worthwhile and the contingency plan as being of value, and whether they are confident to provide the input required to complete the plan. The questions were:

1. Did you attend the workshop?
2. Do you feel the workshop was a good use of your time? (Yes/No)
3. What was the most valuable achievement in the workshop?
4. Do you feel more prepared to face a seabird mortality event? (Yes/No)
5. Do you think the document, when finalised, will help people who were not here today? (Yes/No)
6. Do you think the document should be developed through a different method- if yes, please explain briefly
7. Did we miss some aspect that you would like to add or do you have any comments you would like to make?
8. Are you confident to give input into the plan going forward, to finalise it, and do you know what is needed from you (if anything). (Yes/No)
9. Do you feel that there is someone else who should be providing the information expected from you? (Yes/No)

Ethics approval for the questionnaires was obtained the UP Faculty of Veterinary Sciences Research Ethics Committee and Faculty of Humanities Research Ethics Committee (reference number 26217369 (REC063-22)).

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The results of the questionnaires were summarised by aiming to highlight dominant issues but report on the full range of opinions.

4. Results

4.1. Development of the South African seabird disease outbreak preparedness plan

4.1.1. Workshop

Fifty people attended the workshop in June 2022, 34 virtually via Microsoft Teams and 16 in-person. The biggest groups were representatives of managing authorities, vets and staff from rehabilitation centres and aquaria, and provincial and district Disaster Management and DFFE officials (from scientists to coastal and marine monitors). Veterinary Services, SPCA and police services had one or two representatives. Attendees from outside the Western Cape included aquarium staff from the Eastern Cape and KwaZulu-Natal (KZN), an official from KZN waste management, and SANParks staff.

During the workshop, the decision was taken to extend the scope of the plan to all seabirds and not only coastal species and progress was made with each chapter as follows:

Chapter 1 (Introduction and Background) - recommendations to include information on historical outbreaks and background on the Incident Management System.

Chapter 2 (Legislation and policy mandates) – included additional legislation and international conventions to which South Africa is a signatory.

Chapter 3 (Institutional capacity) - added departments and agencies in other provinces and some relevant organisations and role-players.

Chapter 4 (Risk assessment) - There was good discussion about perceived priority sites and a decision to prioritise based on previous disease outbreaks and high bird density, as well as critical biodiverse areas. Risk factors and risk reduction (Chapter 5) were also discussed in terms of broad disease risk and health.

Chapter 6 (Preparedness) - a thorough list of existing monitoring activities was compiled. There was also a decision taken on frequency of patrols to be recommended for different levels of risk. The discussion then moved to monitoring data and its collection, storage and management and the need for a data manager. The most popular idea was to employ someone via a non-government organisation or through an academic institution and the idea of a post-doctoral project, to pilot the concept, was proposed. Automatic notifications about reports of affected birds, to people required to act (rescue or sample birds) would also assist.

A funding model was discussed briefly. The oiled wildlife model (perpetrators cover costs), SA Maritime Safety Authority (SAMSA) and Benguela Current Convention (BCC) funding were suggested as possible examples to follow but no details were provided.

Chapter 7 (Response) - There was considerable discussion about response to mass mortality events, including possible triggers for response and processes to investigate and determine the cause. Input was also given on aspects of coordination and communication, both between role-players and with the public. There was a suggestion for template media releases, Frequently Asked Question (FAQ) documents and a website with links to all organisations and information. Finally, there was discussion about guidelines for carcass disposal.

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4.1.2. Document preparation process post-workshop

The only contribution submitted in the months after the workshop was a document with euthanasia guidelines from the SANCCOB vet.

After a reminder from DFFE, the SANParks vet submitted some contributions. The input included significant additions to the definitions, information on SANParks structure, some information on a structured disease risk analysis process, and a few comments. No other input was received.

The meeting with SANParks and CapeNature in November 2022 included a discussion of SANParks structure and seabird colony monitoring and its logistical limitations. There was preference for a system of adaptive monitoring and exploring the use of drone surveys. There was a request for clear criteria for closing access to tourists during an outbreak and assessment of the consequences of different actions at different sites. There was also discussion about funding for surveillance and a suggestion that each organisation have someone officially requested by DFFE to report on monitoring activities and data collected.

The latest draft, distributed to the smaller group of key role-players in March 2023, was further populated by referring to role-player organisations' websites for their structure, role and legislation, to literature published about diseases and previous outbreaks in southern African seabirds and to data from BirdLife International on Important Bird Areas (IBAs). IBAs were matched to species of highest conservation status and maps were compiled from data obtained from BirdLife International. Instructions for an early warning system, including monitoring and data reporting were detailed in Chapter 6 (Preparedness). This was based mainly on procedures and documents from previous outbreaks. Considerable content was also added to the chapter on response (Chapter 7), related to outbreak investigation, referring to Preece *et al.* (2017) and Reingold (1998), communication, coordination, incident management, carcass disposal, biosecurity, and vaccination.

The response was better to this more compete version of the preparedness plan, with comments received from four role-layers. However, some input was critical without much constructive contribution. The CapeNature Marine and Coasts Manager, who led the 2021 outbreak response, again suggested that the plan be re-structured to align with the Incident management System but did not describe how. The justification is that it will make the plan more easily understood across different government departments. He also indicated where Standard Operating procedure (SOP) documents would be required.

The Birdlife South Africa Seabird Conservation Programme Manager stated that the IBAs for South Africa are out of date and do not represent important seabird colonies, and that census data should be sourced from DFFE.

The SANParks marine biologist made the most concrete contribution, adding a section in the Background on other seabird conservation pressures, adding names to the contact list and adding a section related to tourists to the biosecurity appendix.

Lastly, the control environmental officer from the DFFE Directorate: Oceans Conservation Strategies added details on environmental management policy and legislation related to seabirds.

A last meeting with the CapeNature Marine and Coasts Manager, in an attempt to plan the integration of IMS, resulted in the resolution that the content would be finalised, and an attempt made to plan a tiered response to mortality events. Experienced CapeNature officials would then be asked to assist with restructuring the plan to align with IMS.

The draft South African seabird disease outbreak preparedness plan, without adaptation to IMS, is included as Annex 4.1. The chapters comprise:

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Chapter 1 (Introduction and background), which includes the scope and objectives of the plan, basic principles of animal disease agents and disease control and historical disease outbreaks in South Africa and background on coastal seabird conservation threats;

Chapter 2 (Constitutional, legislative and policy mandates) lists legislation related to: environmental and wildlife management and protection, animal disease control, animal welfare, disaster management and municipal management and also lists international agreements and conventions;

Chapter 3 (Integrated Institutional Capacity) describes the different stakeholder organisations, their responsibilities and mandates and organisational structure and includes national and provincial government, rehabilitation centres, universities and aquaria, among others;

Chapter 4 (Risk Assessment) includes disease hazards identified by Parsons and Vanstreels (2016), vulnerable species (listed species of conservation concern) and Important Bird Areas;

Chapter 5 (Disaster Risk Reduction) is divided into direct disease prevention measures, related to biosecurity, and general conservation measures to increase resilience of coastal bird populations;

Chapter 6 (Preparedness planning) covers surveillance and early warning and activities and documents that should be achieved to ensure preparedness;

Chapter 7 (Response) includes outbreak investigation guidelines, communication channels and methods (including communication with the public and media), outbreak management including carcass removal considerations, managing sick birds, biosecurity, considerations for vaccination and determining the end of an outbreak;

Chapter 8 (Recovery) covers restocking of equipment and consumables, debrief sessions and resumption of routine monitoring.

4.1.3. Questionnaire results

4.1.3.1. Pre-workshop questionnaire

Twenty-one responses to the pre-workshop questionnaire were received, of approximately 75 people that were sent the link with the documents for the workshop (28% response rate). Five had not been involved with a seabird mortality event before and so did not complete the questionnaire.

The sixteen full respondents reported a variety of roles played during seabird mortality events, most with more than one role. Thirteen were involved with monitoring and reporting, nine with operations on the ground, five with coordination/ management, five in a supporting/ advisory capacity and three with administration and/ or regulatory work.

Thirteen felt that their organisation had been prepared in some way for the mortality event, though one, who said they were not ready, said they were more prepared for the 2021 HPAI outbreak than for 2018. Two of thirteen mentioned previous experience in the organisation or among staff, seven mentioned having some guidelines, protocols, SOPs or plans of some sort for different aspects of the response and four included the word “biosecurity”. One stated that they were prepared with equipment, PPE and “materials” but one said they had “minimal resources put aside for disasters”. One mentioned that identifying a private laboratory assisted with obtaining faster test results.

When asked about where there was least preparation, 7/16 answers referred to the large number of birds affected, 6/16 answers mentioned funding or budget, four mentioned human resources or capacity and three referred to data collection and reporting.

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The biggest challenges again included funding (6/16), five mentioned carcass disposal, five mentioned reporting of cases and maintaining a central communication and reporting route, three talked about maintaining tourism or dealing with members of the public, three mentioned logistics of transporting samples and testing, three mentioned dealing with sick birds and euthanasia and three mentioned maintaining biosecurity or dealing with contaminated materials.

When asked if prior planning would have helped, 12/16 said, “yes”, two said, “no” and two wrote about funding.

4.1.3.2. Questionnaire after the workshop

There were six responses to the second questionnaire, out of 53 people who were emailed the link (11% response rate). All respondents felt that the workshop had been worth their time and that the document would be helpful when finalised. When asked about the most valuable achievement of the workshop, three mentioned liaison, sharing ideas and the collaboration to solve problems and write the document. One mentioned the commitment by all organisations to “regular monitoring and random AI testing as part of an early detection and preparedness response plan”. Another had valued “working through the document chapter by chapter and being able to give some input in a document that will be valuable in the future” and the last said, “It highlighted the different potential challenges that may need to be overcome if - or when - another outbreak should occur.”

All felt the right approach had been taken to draft the plan and 5/6 said, “Yes”, they felt more prepared for a seabird mortality event. However, 1/5 was not confident they would be able to give input going forward and 2/6 felt that they were not the correct person to provide the information requested.

4.2. Development of the South African seabird HPAI protocol

DFFE officials requested input on a draft HPAI protocol from Western Cape rehabilitation centre vets and L Roberts in September 2023. The protocol consisted of the seabird colony biosecurity protocol drafted by L Roberts in 2018, with added sections on clinical signs, disease surveillance and reporting HPAI in seabirds, from various sources. This document was corrected and updated by L Roberts and comments were received from vets at rehabilitation centres.

Another version of this document was circulated to the same group in early March 2024, with added sections on sampling, carcass collection and disposal. These appear to have been sourced from guideline documents compiled by Vet Services and SANCCOB during the outbreaks, and from other international guideline documents. There was a need for a protocol to be available for the new team taking over on Marion Island, with HPAI outbreaks having occurred on other sub-Antarctic islands and in Antarctica.

The SANCCOB vet and L Roberts drafted a final version of the HPAI protocol with the Marion Island team in mind, though retaining the document for general use. The new sections were updated and corrected and re-written to avoid plagiarism of outside parties, where necessary. Detail was added to make recommendations more practical, including specifications for personal protective equipment (PPE) and a section on dealing with live, sick birds was added. Another addition was a table describing a tiered approach to outbreak management, depending on increasing severity and zoonotic risk to personnel. It was based closely on a table from the Government of South Georgia & the South Sandwich Islands’ Biosecurity Handbook, revised October 2023 (and acknowledged).

The final “Protocol for the management of high pathogenicity avian influenza in seabirds” contains detailed information on managing HPAI specifically, rather than disease in general, and starts with information on HPAI transmission and spread, moving on to the risk to humans. It then describes clinical signs of HPAI in birds and processes for reporting suspected cases, as well as specimen collection for laboratory testing and confirmation. It moves on to management of sick birds, making

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decisions about carcass removal and disposal and biosecurity guidelines to prevent virus spread. These include details of processes required, protective equipment and suitable disinfectants. The protocol ends with the table describing the tiered approach to outbreak management.

This final version of the HPAI protocol (included as Annex 4.2) was returned to DFFE with the recommendation that a specific set of recommendations for Marion Island be drafted next, with input from all decision-makers and experts. DFFE requested role-players in the South African National Antarctic Program (SANAP) to give input and requested L Roberts call a meeting to discuss a plan for Marion Island.

4.3. Drafting of the Marion Island HPAI protocol

Having received a set of comments from a Biodiversity Officer, Directorate: Environmental Programmes, DFFE on aspects that would not be feasible or applicable on Marion Island, a draft Marion Island protocol was circulated by L Roberts approximately a week later. All aspects impossible on or not applicable to Marion Island had been removed and the scope was broadened to include monitoring and sampling of marine mammals. Additional information required for the island was also added. The protocol was adapted and focussed for Marion Island by adding extra background for the Antarctic region, and relevant sentences from the Prince Edward Islands Management Plan and revising the reporting structure to align with current structures for island management. Warnings about spillage of potentially harmful disinfectant, and safer disinfectant options were also added, with a comment that decisions were needed on use of disinfectant. The table describing the tiered response was also adjusted and a list of other guidance documents was added, from other international role-players in Antarctica. A seabird and marine mammal sampling protocol was shared by the Latin America Program of the Wildlife Health Center at the UC Davis School of Veterinary Medicine, to which a set of notes was added to provide specific instructions for sample handling, storage and record keeping on Marion Island. This was attached as one of the annexures. The other annexures included a contact list, a supplies list for sampling equipment and protective clothing, a template for recording details of suspected cases, and a draft media statement.

This document was circulated with the invitation to the meeting to discuss HPAI response on Marion Island. Before the stakeholder meeting, preliminary input from researchers with experience on Marion Island included requests for more information on HPAI in marine mammals, a suggestion that one person be responsible for record-keeping and sample storage and a query about guidelines on antivirals for humans. Comments on monitoring and carcass disposal indicated where some aspects would not be feasible. It was also suggested that the South African mainland and Crozet and Gough Islands are the most linked and closest sites that should be monitored for HPAI as part of an early warning system for Marion Island.

A virtual meeting was held on 5 April, with good attendance by DFFE officials and researchers. They were given an introduction to HPAI and the global situation and an overview of the main objectives of the plan and then specific questions were addressed in order to make decisions about the potential response. During the meeting, it was decided that carcass disposal would not be possible and therefore that carcass removal would only be done in certain circumstances where it was expected to have more effect. Euthanasia and any treatment of sick animals was also agreed as contraindicated. There was no perceived need for scheduled patrols to inspect for signs of HPAI, as routine work was expected to cover most sites, but it was agreed that this would be assessed during the planning of the year's work. Little progress was made in agreeing on a clear reporting and communication protocol to ensure accurate updates to all role-players and guidance for island staff.

The agreed changes were made after the meeting, including more information and guidance for marine mammals, sampling and treatment for humans (based on guidance from the National Institute for Communicable Diseases) and a statement about the need for confidentiality until official

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notification of an outbreak had been made. Only overviews of carcass management and handling of sick birds were included and an attempt was made to tailor the biosecurity guidelines further to conditions on the island.

A revised draft was circulated again and final comments were received from three researchers. Concurrently, further work was done to clarify the instructions for sampling and record keeping, also with the input of the researchers. These final documents were submitted to DFFE Oceans and Coasts for final editing and dissemination, on 20 April 2024. The documents are included as Annex 4.3.

5. Discussion

The need for better preparedness was demonstrated during both the 2018 and 2021 HPAI outbreaks in South African seabirds, though recent experience of the 2018 event assisted in 2021. The idea of a written plan was welcomed by all role-players and there appeared to be willingness to assist and ensure that it would address all needs. However, the process to complete it was challenging and much lengthier than expected. The reasons for this have been assessed and recommendations for similar future projects are made here.

The ideal person to lead the drafting of a preparedness plan is someone with knowledge of the process and experience with similar events, but also with time available to dedicate to the task. The alternative would be to employ a professional, without the subject knowledge but with expertise in disaster preparedness and in organising stakeholders to give the desired input. In this case, it appeared that there were few people with time to spare and no-one who felt that the task was completely within their mandate. The wide variety of role-players seemed to make it easy to avoid the responsibility and suggest that it should belong to someone else. It was fortunate in this case that the drafting of a plan was already envisioned as part of a PhD project, so there was someone to take on the task. However, the lack of expertise in preparedness planning was a disadvantage, as was lack of time.

Although L Roberts had a general understanding of disease processes, seabird ecology and conservation and the different groups involved, it was still necessary to have input from the role-players themselves. This was required to be able to provide details of organisational structure but also operational aspects of managing seabird colonies. Involving role-players in the planning process should also assist with a wider understanding of and awareness about the contents of the plan. However, gaining the input from all these people was a challenge. The workshop was probably the most productive engagement and very little other input was received until the plan was much more complete.

It would probably have been better to start consultation based on a more complete document, clearly marking areas of uncertainty, and to aim specific questions at specific people, ideally in a focussed discussion. This approach was taken with the HPAI plan for Marion Island and more constructive feedback was received, though a sense of urgency probably helped. Asking people to write sections of the preparedness plan, even when assigning specific sections to specific people, was largely unsuccessful. This may have been related to a feeling by some of the workshop participants (2/6 who responded to the post-workshop questionnaire) that they were not the right person to ask for input. Providing a document for correction and additions was more productive. This could be because there were then clearer examples of what was needed. However, the type of role-player may also influence the quality of the input. Academics or researchers familiar with reviewing and contributing to documents may be more helpful, depending on their other commitments, whereas technical field staff are far more unlikely to write anything at all.

It is also important to create the right circumstance and environment for stakeholders to contribute meaningfully. Requesting dedicated time, at a venue separated from their normal duties, and gaining

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support of their managers, may be a better approach. The feedback received on the workshop, via the questionnaire, was positive. However, the sample may have been biased towards people who had had a positive experience and were then more likely to complete the questionnaire. Shmueli *et al.* (2021) discuss reasons for reluctance to take part in community-based disaster planning and cite lack of willingness to spend time that is required for more urgent tasks. Creating dedicated time for planning, arranged in advance, may be a way to navigate this. More regular, smaller meetings, with specific questions to answer and decisions to make, would probably have been more productive.

Lack of written participation by other role-players was probably also due to timing. The need for a plan is recognised most clearly during and immediately after an emergency and possibly immediately before, if enough of a warning system is already in place. There is unlikely to be time available during an event, unless someone can dedicate themselves to documenting actions taken and decisions made, and information that was required and sourced. Realistically, the best time to achieve good participation in planning is immediately after a similar event, while the memory and urgency of it are still clear. If an attempt will be made to plan before an event has occurred, the sense of urgency is likely to be lacking. The workshop was conducted relatively soon after the 2021 outbreak but the urgency probably became less over time.

If there is an opportunity to plan before an event occurs, it is challenging to decide on actions without information about the situation (Shmueli *et al.*, 2021) and without the pressure to make a decision. One does, however, have to be realistic about how well one can plan and how accurately one can predict what will happen. The best that can be achieved may be ensuring that all potential role-players discuss the matter, that their contact details are listed for later discussions, and that any information that is currently available is gathered and made accessible for when the event occurs. An attempt should be made to describe the factors that will affect a decision and to provide instructions to ensure the correct information is gathered to be able to make the decision, when required. An easy trap to fall into is believing one has to plan for every situation and eventuality. Once the risks have been described and the available response options have been listed, it should be relatively simple for managers to formulate plans for specific sites, where some risks and options will fall away. This was demonstrated by the rapid drafting of the options for Marion Island. Alternatively, a best guess must be used, based on available information, for the sake of having a decision in advance. This is difficult but may be necessary.

It has been very helpful to have other, similar plans to work from when compiling a first draft; it was more difficult when such plans were less common. Designing a tiered approach to an HPAI outbreak in seabirds, to allow planning for both minor and major events, appeared particularly challenging, but referring to an existing similar document (Government of South Georgia & the South Sandwich Islands' Biosecurity Handbook) made it much easier. Permission to re-use and adapt should be sought and full acknowledgement should be made, but free sharing of information and documents should be general practice with challenges of such global importance. The general structure of different organisations' documents should probably be similar, covering similar topics and providing similar background to the disease in question. However, the specific legislation, government structures, reporting routes, non-governmental organisations and division of responsibility, will differ between countries and possibly regions within countries. Collaboration from all stakeholders is required to ensure the plan is complete. For example, the Scottish Wild Bird Highly Pathogenic Avian Influenza Response Plan (Scottish Government, 2023) is subdivided in a very similar way to the South African seabird disease outbreak preparedness plan, though it has more avian influenza specific information. Advice for record-keeping and carcass management do not tend to differ much. However, the Scottish plan, for example, quotes a risk assessment that the government commissioned, that concluded that there was no evidence that carcass removal reduces virus spread (Adam *et al.*, 2023). Their default advice is therefore to leave carcasses in place, whereas the South African HPAI protocol states that carcass removal can be done if resources allow it.

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Another difficulty is planning how to fund disasters and emergencies. Commitment to this has been non-existent so far, with regards to planning for seabird mortality events in South Africa. Shmueli *et al.* (2021) list funding as a common barrier to preparedness planning and, in South Africa at least, government budgeting systems do not seem to accommodate funds where there is uncertainty about if or when they will be used. It is possible to purchase equipment and consumables that can be kept in reserve, but reserve funds are more difficult. Possible preparation that can be made is to explore and document the process to obtain emergency funding within an organisation, and to list organisations that may assist in emergencies.

Once the plan is drafted, finalised and adopted, the continuing challenge is to ensure that role-players remain aware of and familiar with it and that it is reviewed and updated when necessary. Realistically, no one is likely to read the plan voluntarily in peacetime and anyone who does is unlikely to remember much. Regular refresher training and simulation exercises are therefore vital to ensure that role-players are familiar with the document and that it achieves its goals.

This plan has been successful in summarising the potential role-players and the legislation under which they function but is still lacking a structure with which role-players are comfortable. Re-structuring in line with IMS may help, as many of the field staff have had the IMS training and use the system for responding to other emergencies, like wild fires. Another shortcoming is that outdated Important Bird Areas were used to prioritise areas for monitoring. Sites such as Stony Point Nature Reserve, housing important seabird breeding colonies, are not included. However, most breeding sites affected by HPAI from 2018 to 2024 are shown and the current map and table is still useful.

Except for Marion Island, there is also still a lack of specific instructions and SOPs that will provide field staff with more security. A high priority for disaster preparedness should be ensuring that field staff are prepared for the initial signs of the disaster. They should have sufficient awareness and training to avoid making mistakes (e.g. spreading a disease further) while the situation is still being assessed and the response is being activated. They should be also able to gather the correct information to assist with assessments and to inform the correct people, who will be able to start discussing options and making decisions.

Once this plan is finalised there will also be a need to ensure it is approved by national Veterinary Services. There is also a need for preparation for HPAI in marine mammals and identification of role-players willing to take responsibility.

6. Conclusions

Though preparedness planning is generally agreed to be good practice, it is not necessarily easy to achieve it and to obtain input from knowledgeable stakeholders. This project ensured that progress was made with contingency planning for disease outbreaks in South African seabirds, that guidelines for managing HPAI were collated and that some level of preparedness was achieved, in case of a potential HPAI outbreak on Marion Island. However, the process was slow, both because of a lack of dedicated project leader and probably because of a flawed approach. It is likely that too many people were involved too soon and there should have been less reliance on written input and more time spent in discussion, in smaller groups, once a first draft was complete. However, compiling a first draft can be extremely challenging without other plans as examples and without knowledge of future events. Sharing of plans and protocols and discussion between parties working on similar projects is strongly encouraged to avoid wasting time and effort.

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8. List of Annexes

Annex 4.1- South African seabird disease outbreak preparedness plan

Annex 4.2- Protocol for the management of HPAI in seabirds

Annex 4.3- Protocol for the management of HPAI for Marion Island

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Chapter 7
Discussion and Conclusions

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1. Discussion

1.1. Clade 2.3.4.4.b HPAI viruses and their global impact

High pathogenicity avian influenza, Goose-Guangdong (GsGD) lineage viruses re-emerged as a global threat to poultry health in approximately 2014 (The Global Consortium for H5N8 and Related Influenza Viruses, 2016). The clade 2.3.4.4 viruses have since diversified genetically and in host range and pathogenicity, and spread to every continent except Oceania by 2024. Clade 2.3.4.4b H5N8 viruses caused concerning mortality in poultry, waterfowl and some seabird species in Europe and Africa, including South Africa, from 2017 to 2019 (Abolnik *et al.*, 2023; EFSA *et al.*, 2023; Harvey *et al.*, 2023). However, the clade 2.3.4.4b H5N1 viruses that emerged in 2020 have surpassed H5N8 in individual animals, species and continents affected and are a recognised conservation threat (Ramey and Handel, 2022). Millions of poultry have been lost, seabird populations have been decimated, and thousands of marine mammals have died in South America (Campagna *et al.*, 2024). The virus has also reached Antarctica and threatens the millions of seabirds breeding there in dense colonies (WOAH, 2024). These outbreaks in wildlife are challenging to monitor and respond to and available mitigation measures are few. It is essential that the experiences and lessons learnt are shared and that careful observations are made, to understand the epidemiology of the virus as far as possible and allow for potential appropriate prevention and mitigation measures to be identified.

1.2. HPAI epidemiology in South African seabirds

Observation of HPAI outbreaks in South Africa, described in Chapter 3, have most of all highlighted the variable and currently unpredictable effects of the virus. It has been detected in all four coastal seabird groups (penguins, gannets, gulls and terns), and infected all four endangered species (African Penguins, Cape Gannets, Cape Cormorants and Bank Cormorants) though it has had varied effects. The two different clade 2.3.4.4b H5 viruses appear to have had different species predilections, though this could be related to environmental factors or to immunity conferred by the first virus that protected against the second. The latter would point to the H5N8 virus having spread more easily in some species than others. Another explanation is that Swift Tern densities were lower after 2018, either due to mortality during the H5N8 outbreak or different behaviour, so the H5N1 virus did not affect them in the same way in 2021. Alternatively, the virus was not present during the 2021 or 2022 Swift Tern breeding season, when most aggregation occurs, and the Cape Cormorants were unlucky enough to provide the ideal situation for rapid transmission that year.

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It is also interesting that although the first detections of H5N8 and H5N1 viruses in the Western Cape were in different seasons (late winter vs late autumn) it took approximately two months for seabird-specific viruses to emerge, in October 2017 and July 2021 (SA13 genotype) respectively, and another two months to start causing significant mortalities (Abolnik *et al.*, 2023; Peyrot *et al.*, 2022). It is also curious that, according to limited sequencing data, two different genotypes may have caused the 2021 mortalities in Cape Cormorants on the West Coast (SA13) and at Dyer Island, on the South Coast (SA4). How and why the SA13 genotype persisted into 2022 and 2023 requires further investigation (Abolnik *et al.*, 2024, 2023).

It is reassuring that the severely affected sites have not been affected in the same way in subsequent years. This may be due to widespread immunity of birds which had been subclinically infected, or may be due to reduced colony density after outbreaks. However, the reason that the Boulders colony was largely spared in 2021 and had relatively high mortality in 2022 is still unknown. Possibly, there was an unusually large number of birds breeding on Foxy Beach, where most cases were found, that year.

HPAI outbreaks in Africa Penguins between 2018 and 2022, first due to the H5N8 virus and then H5N1, demonstrated that the disease can cause high mortality in the species. However, some colonies only had a few reported cases. This study did not find any evidence of prior immunity at colony level, so this is unlikely to explain the apparent lower susceptibility in some colonies. The highest numbers of mortalities recorded were on Halifax Island in Namibia (2019) (Cannon, 2019), on Dyer Island (2021) and in Simon's Town, from the Boulders colony (2022). High doses of virus from the nearby, badly-affected Cape Cormorant colony on Dyer Island, and high nest densities on Halifax Island and Foxy Beach, Boulders, may have contributed to the severity of the penguin outbreaks. Dassen Island has the lowest nest density and no penguin cases were recorded for either outbreak, but it is also the most difficult site to monitor thoroughly. Colony size and density also appeared to have affected the Swift Tern outbreaks in 2018 and other authors have made similar observations overseas (Alexandrou *et al.*, 2022; Gaidet *et al.*, 2012; Maxted *et al.*, 2012). High virus doses may overcome immune defences that can cope with lower doses and high nest densities will increase transmission rates through close proximity of birds, possibly severe environmental contamination, and the prolonged time that susceptible birds are exposed to infected neighbours. Sites with large and (or) dense seabird colonies and multiple species should be prioritised for disease monitoring and preparedness planning, considering all possible mitigation measures.

Most of the sampling for the African Penguin colony survey, described in Chapter 4, was done before the worst of the 2021 outbreak, but sampling of birds admitted to SANCCOB continued and there were very few detections between mortality peaks. Though the sampling at SANCCOB was biased, because healthy birds are not usually brought to rehabilitation centres, it can also be said to be targeted, with the highest likelihood of sampling a sick penguin for the least amount of effort. The low antibody detection rate in African Penguin colonies argues against adult penguins maintaining HPAI virus subclinically between outbreaks. There was also only evidence of previous exposure in 1% of sampled birds, which indicates a lack of pre-existing protective immunity. Because colony sampling in the Western Cape mostly preceded the worst 2021 outbreaks, the number of subclinical infections during outbreaks is still unknown. From the confirmed positive cases in 2018 and 2021/22 and the sampling of penguins taken to SANCCOB for rehabilitation, it seems that chicks are less likely to be infected than adults. However, if it was rapidly fatal in chicks, they may not have been admitted and the ages of affected penguins on Dyer Island were not available. The outbreak at SANCCOB in November 2022 demonstrated that chicks can have subclinical or mild infections. However, it is possible that the artificial high density of and close contact between chicks caused a higher proportion to be infected than would be in the wild. Alternatively, good nutrition and protection from the elements may have led to more mild signs and lower mortality than in the colonies. Both the seropositive birds found in the colonies and the two mild infections in adults at SANCCOB demonstrated that adults can also be mildly affected, but these were still in the minority of positive penguins.

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In contrast with age predilection in penguins, the H5N8 virus appears to have decimated the chick crop in two Swift Tern colonies in 2018 and at least 900 Cape Cormorant chicks are suspected to have died from the H5N1 virus at the Stony Point colony (described in Chapter 3). There were also high mortalities in tern chicks in Europe (Knief *et al.*, 2024) and in juvenile Great White Pelican chicks in North Africa (Beyit *et al.*, 2023; Lo *et al.*, 2022). The reason for this is not clear. It is generally agreed that although not desirable, losing chicks is preferable to losing breeding adults. Seabirds only breed at a relatively advanced age, many chicks do not survive to breeding age, and older birds gain experience and are more successful at raising chicks (Becker and Bradley, 2007). The loss of these birds seriously retards conservation efforts.

Other possible contributing factors to AIV infection in penguins, identified in Chapter 4, were poor body condition and sex, males appearing more likely to be infected, but females more likely to have antibodies in the colonies. Small numbers of affected birds may be the reason for the lack of statistical significance, so this should be investigated further, where possible. The apparent predominance of females being admitted for rehabilitation and males sampled in Namibia should also be noted for further research.

Further research will be required to better understand the epidemiology of HPAI, in other species, possibly with novel viruses, and also in the ecosystem as a whole. Properly planned surveys will be necessary but ideal sample sizes may have to be compromised if endangered species or delicate ecosystems are involved, as was found in this study. It will be vital to examine any available data that may indicate how research can be targeted and prioritised. The cost of seabird disease research, in terms of equipment, transport, consumables, time and human resources is also considerable and is another reason that prioritisation is important.

1.3. HPAI outbreak response in seabirds (Chapter 3)

South Africa, and the Western Cape province in particular, experienced some of the first HPAI outbreaks in seabirds, before the virus became an internationally recognised conservation threat. As a consequence, there were few publications about established best practice and it was frustrating to try to respond appropriately. At that time decisions were made based on basic disease control principles and limited information about the epidemiology of the disease. Accounts of experiences and lessons learnt in South Africa appeared to be helpful to others who experienced similar outbreaks later, which was rewarding. However, it has also been reassuring, and simultaneously discouraging, to find that few other solutions or effective response measures have been developed. Better resources have allowed risk analyses and assessments in other countries (Adam *et al.*, 2023; Rijks *et al.*, 2022). However, the results are not conclusive, if not contradictory. Advice on best practice for a specific situation usually still begins with “it depends”, but there are now at least good descriptions of factors to consider when making decisions about potential activities, such as carcass collection (CMS FAO Co-convened Scientific Task Force on Avian Influenza and Wild Birds, 2023). Additionally, some aspects are common to all disaster responses, like effective communication. It is vital to ensure that all stakeholders and role-players are aware of the event and their roles, and that they understand what is required. It is very helpful to be able to contact the right people to tackle challenges as they arise. A central coordinating body or person, experienced in incident management and with sufficient authority, is an also advantage.

Besides the management of infected birds, another challenging aspect is the monitoring of outbreaks in order to report with some accuracy. Counting affected birds is challenging but there have also been varying levels of reluctance to report un-tested birds as affected, at least initially and to the World Organisation for Animal Health (WOAH) (Klaassen and Wille, 2023). There may also be a different approach between seabird stakeholders and the national veterinary authorities reporting to WOAH. In South Africa, we decided to count every dead or sick bird as a case, as long as there was no other obvious injury or disease and the virus had been detected in that species and (or) in the area. Where

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only a low proportion of samples tested HPAI positive, as with Cape Gannets, more caution was used, though some parties thought the caution unjustified. Other international groups seem to have used a similar approach, at least for scientific publications (Caliendo *et al.*, 2024; Pohlmann *et al.*, 2023). However, there is still data missing about the numbers of animals affected in some places and post-outbreak censuses will be particularly important there.

The process of gathering data on morbidity and mortality can be very labour-intensive and there are challenges from observation and counting all the way to collating the data for an area or country. It may be difficult to access all affected areas, as well as to account for birds counted more than once in populated areas (Avery-Gomm *et al.*, 2024). Members of the public can assist, but ensuring that the correct data are collected and reaches the right database requires good coordination and communication. Standardising data collection and reporting should help to ensure that important information is collected and assist with accelerating collation and summarising of the data. A digital data collection method, that allows the automatic recording of location and date, and attaching of photographs to support species identification, has the potential to eliminate many errors and to save time, as well as allow automated production of maps and charts. However, despite such a tool being available to conservation personnel in South Africa, the adoption rate has been low and few people use it. It appears to be used more when seabird HPAI outbreaks occur, so may help illustrate peaks in mortality, but the envisaged role of helping to establish baseline mortality and detect early escalation has not yet been realised. The reason for this seems to be that there are already other digital applications or established data collection methods in use. The reporting tool may be seen as the duplication of a task, or an inconvenient addition to already onerous record-keeping. When managing seabird mortality dominates daily tasks, the tool appears to be used more readily. More consultation with field personnel will be required, to understand their current duties and record-keeping. Training may also help to improve the quality of data collected by current methods and reduce the need for data cleaning. Where there are existing databases or data collection tools, exploring ways to import seabird morbidity and mortality data from different sources into one database may be more successful than trying to collect the data using only one method. The current tool could perhaps be adapted for members of the public who do not routinely record data in bird watching or other applications. However, there will also be a need to collate information from existing bird-watching and animal stranding tools and perhaps request modifications that allow vital data to be included. Reporting of surveillance effort (distance or area surveyed) would also help with establishing accurate baseline mortality rates and assessing coverage of the coastline. This is most easily done through a smartphone application that records the route that was travelled.

Currently, there is no practical way to stop HPAI virus spreading, locally or globally, and to protect vulnerable species. The exception may be small populations that are kept in captivity, behind mechanical barriers, and even that has proven ineffective for many poultry farmers. Feasible response measures for HPAI in seabirds are currently limited to controlling human behaviour, to mitigate the risk of human infections and mechanical virus spread, and to removing infected birds as a source of virus. Realistically, however, these measures are unlikely to prevent many wild bird infections overall. Preventing a global human pandemic and transmission to poultry is a far more realistic goal for biosecurity than reducing the infection rate in wild birds. This is important to realise when planning a biosecurity protocol. Resources should be allocated carefully, according to clear objectives, and with realistic expectations about outcomes. In some circumstances, it may be better to use resources to study virus distribution and prevalence and try to understand the epidemiology, to be able to focus response efforts more effectively. It may be difficult to persuade managers that this is a more useful approach, but if nothing can be done to stop an outbreak, the next best action may be to study it and learn.

The limitations to available response options at least make it possible to plan and prepare for HPAI outbreaks, within the framework of legislation, role-players and resources and with guidelines about

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best practice now available (Described in Chapter 6). Routine surveillance and record keeping should allow early detection of outbreaks and allow time to implement plans. The response should be scaled to mortality rate, length of outbreak and zoonotic risk of the virus. Scale will affect resources required, including personnel, and, for example, the carcass disposal method. However, based on the average number of birds frequenting a site, knowledge of the local area and the resources available to the staff, it should be possible to plan for different sized outbreaks. It is then important to ensure that staff are familiar with the plan, and understand which role-players should be involved and kept informed, and what are the appropriate response options. Specific instructions for each site should be compiled but the general guidelines should be provided at a higher level, incorporating legislation and roles of different government and non-governmental bodies. We have found the drafting of this high-level preparedness plan very challenging. Obtaining input from all role-players and finding the time among everyday tasks has been difficult. However, more rapid progress would probably have been made if a more complete plan had been shared at the first stakeholder engagement and more, smaller workshops were held.

Rehabilitation centres have played an important role in South Africa's response to HPAI in seabirds, providing invaluable assistance in sampling and testing, communication, coordination and other aspects (Described in Chapter 3). SANCCOB sourced funding for testing and provided personnel to perform sampling, which would not have been possible otherwise. Limited government and other research funding would not have allowed nearly so many laboratory tests and confirmation of infection in new species and sites. Sampling at a rehabilitation centre, with suitable facilities and trained staff, is also far more convenient than trying to sample in the field. Many seabird colony managers had pre-existing arrangements for SANCCOB to examine carcases, which also assisted with logistics. The rehabilitation centres' existing networks of volunteers, conservation authorities, researchers and other stakeholders were also helpful. This assisted with requests for assistance, communicating with the public and private veterinarians and performing training for conservation field staff. Established and well-recognised rehabilitation centres can also assist with attracting additional funding, collaborators and expertise. Rehabilitation centres provided an extra challenge in terms of infection and outbreak management. Ultimately, decisions about whether to place a rehabilitation centre under quarantine and restrict their activities were based on the balance of risk at the time. If there was high mortality in the wild and few detected cases in the centres, the centres were not perceived as a significant risk to other birds. The risk was in the potential for virus to enter the centres and cause outbreaks, so it was in the centres' interest to implement good biosecurity and prevent this. It is not feasible to reduce the risk of virus introduction to zero however, especially since it's not possible to test every bird on admission. More affordable and accessible test methods may assist with this but are not likely to be available soon in South Africa. In contrast, the outbreak at SANCCOB in November 2022, when there was a low incidence of HPAI reported from the wild, may have posed more of a risk to birds outside the centre and those being admitted. Quarantine was then implemented. This incident provided another valuable learning opportunity, however, both about the likely effectiveness of proper biosecurity and complete functional separation of birds within a facility, and the value of extensive testing to measure the problem and adapt the response accordingly.

Attempting to prevent entry of virus into rehabilitation centres posed a significant logistical challenge. It involved maintaining extra facilities to accommodate quarantine periods, separating groups of birds within those facilities and ensuring clinical signs were detected. It was very resource-intensive. Pen-side antigen tests, like lateral flow devices, may have made the screening process shorter and easier but their use is not permitted currently in South Africa, their sensitivity may be insufficient and the cost may be considerable (WOAH, 2021). The sensitivity of clinical assessments on admission should also be compared to that of the available pen-side tests. Most African Penguins that tested AIV PCR-positive on admission to SANCCOB during the survey were considered suspect for HPAI at the time of sampling, based on clinical signs. The exception was one chick (of the twelve birds that tested positive on admission), admitted as part of a large batch. It was either showing few clinical signs, or was not

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properly observed, maybe due to time constraints. Pen-side tests may have a role to play in such situations where there are possible subclinical or mild infections or limited time for clinical observation. However, subclinically infected birds may shed virus more intermittently or at low levels and the test may still miss infections. Additionally, the cost of screening such large numbers of birds may not be justified if more careful examination and (or) quarantine procedures are possible.

1.4. Vaccination of wild seabirds against HPAI (Chapter 5)

Given the lack of other effective options to protect wild birds, vaccination warrants a structured assessment in every situation, ideally before there is any threat of an HPAI outbreak. A conclusion on whether or not to vaccinate wild birds may be reached rapidly, but should be clearly justifiable. Decision analysis tools and modelling methods may be helpful: there are many interdependent factors to consider and weighing of potential benefits and harmful consequences is essential (Hemming *et al.*, 2022). The aim of vaccination must be clear, to set minimum requirements for vaccine effectiveness (Barnett and Civitello, 2020). Available vaccines are not completely effective and impart immunity for a limited time, but also will not be equally effective in all species. We have some data available for different species, for birds vaccinated with inactivated vaccines in zoos (Osterhaus *et al.*, 2007) but further trials will probably be required.

Planning a vaccination program for HPAI in seabirds will ideally be based on better information about the mechanism of disease spread in colonies. The relative importance of penguins and of their different age groups, and the role of other species in HPAI virus epidemiology is still unknown and will dictate how a vaccination plan should be structured in that species. Vaccination of a key species or age group could play a disproportionately large role in preventing the spread and (or) disease and make vaccination more affordable and feasible (Barnett and Civitello, 2020). The age of first infection and (or) most susceptibility to disease are important factors to consider. Aiming to vaccinate after the waning of maternal immunity, but before initial infection, and possibly most virus shedding, could be effective at reducing chick losses and virus spread, but it may be very difficult to time correctly (Bourret *et al.*, 2018; Wobeser, 2002).

Theoretically, it would be best to identify and vaccinate whatever species is responsible for most virus shedding and dispersal. If, for example, the Egyptian Geese that nest in seabird colonies are responsible for introducing the virus and their immunologically-naïve offspring act as multipliers (van Dijk *et al.*, 2018), maybe vaccination of those offspring will block a major source of the virus. If adult penguins are the primary source of virus for each other (with small numbers of pinpoint introductions from other species), then perhaps maintaining a certain level of immunity, among adults only, would be effective, and annual vaccination of chicks would not be necessary (if immunity is of long enough duration). It may be possible to start modelling these factors for specific scenarios, including areas of uncertainty, and to pinpoint the most influential variables (Bourret *et al.*, 2018; Cleaveland, 2009). This would help with prioritising research and possibly planning targeted vaccination that will be most effective with the fewest harmful repercussions.

If vaccination is deemed infeasible, the barriers should be identified and assessed so that the feasibility can be reconsidered when, for example, new vaccine technology or other resources become available. Vaccination of wild birds with inactivated avian influenza vaccines seems impractical in most situations, given that they would need to be captured and handled individually for both a first and second (booster) inoculation, and given the likely losses due to the stress of capture and handling (Swayne *et al.*, 2014). A wide range of vaccine technology has already been investigated in poultry and the plant-produced virus-like particle vaccine, investigated here, exemplifies some of the characteristics that may be needed for a vaccine for wild birds. If the safety and duration of protective levels can be confirmed, the VLP vaccine would appear to be a more feasible option. Although it still requires individual injection of birds, a single dose may provide sufficient protection during the peak of an epidemic, as an emergency measure, and involves far less expense, effort and stress to the birds

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than two doses of inactivated vaccine. However, mass vaccination with injectable vaccines still poses a serious logistical and ethical challenge, so research into and development of vaccines that can be applied safely in droplet or spray form, via the ocular, nasal and (or) oral mucosa, should still be investigated (Calzas and Chevalier, 2019). Even if a mucosal vaccine had to be applied to each individual bird, it would still be easier and require less expertise and training. The ideal would be mass vaccination via close-range spray or misting, however, planning for suitable climatic conditions could be a challenge.

Mucosal vaccination also offers the chance to induce better protective immunity by stimulating secretory Immunoglobulin A (IgA) that will act against viruses at their natural entry points (Nurzijah *et al.*, 2022; Swayne and Kapczynski, 2008). However, there are challenges to overcome, to ensure that the vaccine antigens reach the required immune cells without being destroyed or removed by the innate immune system. Suitable adjuvants need to be added to VLPs, or additional proteins expressed, to allow VLP vaccines to be effective with mucosal application (Calzas and Chevalier, 2019). For example, intranasal administration of plant-based H5 protein with a mucosal adjuvant ((3',5')-cyclic dimeric guanylic acid) has been found to be successful in preventing death in mice (Major *et al.*, 2015). Live recombinant vaccines are an example of another vaccine technology that may be effective as a mucosal vaccine. The Harderian gland is an area of lymphoid tissue in the paranasal area of birds and in chickens it is an important site of IgA-producing cells. The gland drains into the conjunctival sac and from there into the nasal cavity via the lacrimal duct. This allows immunisation of birds via an eye drop. The "common mucosal immune system" should allow the development of mucosal immunity in the gastrointestinal tract also, even though the vaccine was applied to the respiratory tract (Swayne and Kapczynski, 2008).

Colonial-breeding seabirds such as African Penguins may pose less of a challenge to vaccinate in the wild than some mammals, and birds that have more dispersed nests. Flightless African Penguins especially are easier to catch in and defending their ground nests than many other species. Aggregations of nests may also make vaccination of chicks relatively easy. However, the effect of colony disturbance, causing stress and disruption, will be weighed against the benefits expected from a vaccination program. If nests are abandoned, it leaves chicks and eggs vulnerable to unfavourable environmental conditions and predators. Breeding attempts could also be abandoned. The potential effect of HPAI on a breeding season, and the protective effect of vaccination, must be carefully weighed against potential losses due to the campaign itself. Certain categories may be inaccessible (e.g. fledglings out at sea) or need to be excluded, e.g. penguins undergoing their annual catastrophic moult. Moulting birds were not sampled in this study, due to their perceived susceptibility to stress and over-heating (Webster *et al.*, 2016). During this phase, all feathers are lost and replaced over approximately two weeks and the birds cannot swim to hunt in this time, as they are not waterproof. Pre-moult birds in this study gained at least 22% extra body mass, compared to day 0. Studies show they become slightly anaemic (Mazzaro *et al.*, 2013; Sarran *et al.*, 2006), have high thyroxine (T4) and triiodothyronine (T3) and low sex hormone levels (Otsuka *et al.*, 2004) and one study found low corticosterone levels (Mazzaro *et al.*, 2013). These factors may not affect immune response to a vaccine but moulting birds being stressed and disturbed and being chased into cold water may be an unacceptable side-effect of a vaccination program.

Vaccination may not be deemed necessary or feasible until the population is much smaller. Though this is obviously undesirable, and would mean that conservation efforts had failed, decisions and vaccination programs may become easier. It may also be easier to obtain the required regulatory approvals from veterinary and conservation authorities and to source funding for the program, which will be significant hurdles for any vaccination program. The possible consequences of vaccination for the evolution of the virus, disease control and trade barriers will be important considerations for veterinary authorities. Depending on the vaccine chosen and its, and other HPAI vaccines', approved use in poultry, it may be necessary to keep tight official control over the vaccine supply, assuming its

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use is approved in seabirds. Spray vaccines and likely vaccination of unintended targets could be particularly difficult to get approved. Additionally, conservation authorities need to be convinced that the value of vaccination outweighs the disturbance to the colonies, the cost and the manpower required, even if they are not entirely responsible for providing the latter two resources.

One last aspect to consider is how post-vaccination monitoring and virus surveillance will function. It will be necessary to establish whether the vaccination program is achieving its goals and if it is causing harm (Cleaveland, 2009), such as driving virus mutations, mechanically spreading virus or disrupting breeding in seabird colonies. Current HPAI detection in South African seabirds relies heavily on clinical signs and does not yet have any structure or overarching plan. Funding for a surveillance plan will be one challenge and ensuring adequate coverage of the correct species will be another. Again, better knowledge of the disease epidemiology will be required. However, depending on the species and numbers vaccinated, it is possible that viral escape mutations and their detection need not be a concern in seabirds. Considering that there are only approximately forty thousand African Penguins, even if all were vaccinated, the viral evolutionary pressure would likely be insignificant compared to vaccinating a hundred thousand layer hens on the average Western Cape farm. Additionally, virus circulation in other, unvaccinated, probably more epidemiologically important wild bird species, is likely to dilute the effect of relatively few individuals. Vaccinating species that play a key epidemiological role may need closer monitoring, but, then again, higher evolutionary rates have so far been observed in viruses circulating in countries with vaccinated poultry (Cattoli *et al.*, 2011), which probably dramatically outnumber any wild bird species. There are billions of chickens on Earth (Bennett *et al.*, 2018), that account for a biomass of at least double that of all other birds combined (Bar-On *et al.*, 2018).

2. Final Conclusions

Since 2018, South Africa and many other countries have rapidly gained experience responding to clade 2.3.4.4b high pathogenicity avian influenza outbreaks in seabirds and other wild bird species. Aggregation of seabirds for breeding makes them vulnerable to HPAI infections, but many species are also already endangered and are severely impacted by the loss of mature breeding adults. Adult African Penguins appear to be more susceptible to HPAI than juveniles and this may translate into more serious and long-term impacts than if younger birds, in which less had been invested, were affected.

Although there is no way yet to guarantee the protection of a seabird population, there are now guidelines on appropriate measures and on monitoring and reporting, as were written for South Africa as part of this dissertation. It is possible and necessary to plan for HPAI outbreaks, and this should be done well in advance, as the collaborative process required can be slow. Effective planning should allow more efficient use of resources and for more advances to be made in understanding HPAI virus epidemiology. It should also be applied by the relevant role-players to prepare for outbreaks in other vertebrate groups.

A better understanding of HPAI epidemiology in different species, and the role of environmental contamination specifically, may open up further management options or allow prioritisation of resources. As African Penguins to not appear to be a subclinically-infected source of virus for other species, but more a victim, research and control efforts could rather aim to identify and manage more epidemiologically important species. Resources may also be required to do further surveillance in healthy birds, to understand how the virus is being maintained, especially the SA13 seabird genotype in South Africa. The reservoir species are unknown, as are the important transmission routes, between and during outbreaks. Serology may assist, but the accuracy of the test used, in the species to which it will be applied, should be established first. The risk factors identified already, and supported by this work, like colony density, should also be considered in terms of mitigation measures. General

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conservation efforts, for example protecting and augmenting space for breeding, may also help to protect against severe HPAI outbreaks.

This dissertation illustrates that vaccination of a seabird species, the African Penguin, could be a safe and effective preventive measure, though further improvements in vaccine technology may be required to overcome some of the logistical challenges. Vaccination of seabirds should be considered in all situations, preferably through a structured process, as there are many interdependent factors to consider. Vaccination may not be a feasible option for a particular population, with vaccines and resources currently available, but the barriers should be identified so that an opportunity to implement vaccination in future is not missed.

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Annex 1

Report: results of sampling seabirds for avian influenza at SANCCOB on 12 December 2022

Seabirds at SANCCOB were sampled on 12 Dec 2022 to estimate overall HPAI virus prevalence and to compare prevalences between epidemiological groups.

Aims: Assess whether actions to prevent virus spread were working and whether removing suspected cases and placing them in quarantine areas was worthwhile.

Additionally, estimate the sensitivity of "cloudy eyes" as indicators of virus shedding.

Methods

Almost all of the birds in the centre were sampled and pooled samples were tested to calculate prevalence. The sample size required to calculate prevalence from individually tested birds would have resulted in more tests and therefore a higher cost.

Sampling on 12 Dec excluded most of those African Penguins quarantined in the Wash Bay quarantine area, which were sampled 5 Dec (lab ref 704741). Only 1/10 pools had tested positive, which gives an estimated prevalence of 0.02 (95% confidence interval 0.1; 0.09), assuming perfect tests. All birds in the positive pool had been moved to ICU 2 on 9 December and were sampled individually on 12 Dec. The birds in the Home Pen were not sampled.

The data analysis "method uses generalised linear modelling to calculate maximum-likelihood estimates of prevalence and confidence limits where multiple different pool sizes are used." The assumption of a perfect test may not be completely correct. The published sensitivity of the commonly-used VetMAX™-Gold AIV Detection Kit is 99% for chicken samples and the specificity is 100%, but virus can be damaged by poor storage conditions, lowering sensitivity and any cross-contamination of samples will lower specificity. However, the tool is adequate to compare relative prevalences.

78 pools were tested: 74 from African Penguins and 4 from other species (Hartlaub's and Kelp Gulls and a heron) as well as 7 birds tested individually: Cape Gannet G126 kept with the ICU group, AP787 who became a suspected case on 12 Dec and had been in Pen 8, and the 5 penguins that had been part of the positive Wash Bay pool, sampled 5 Dec.

Records of suspect cases (with "cloudy eyes') identified after 12 Dec (and placed in the Education Room) were compared to the test results from all groups except ICU 2 (quarantined). The probability of there being a suspect bird that contributed to a positive swab pool was then compared with that for suspect birds contributing to negative pools (Relative Risk or Risk Ratio).

Results

Both the gannet and AP 787 tested positive, as well as 14 of the pools (Lab references 705340 and 705565). However the other ICU pools were negative, including the gulls and heron in the aviaries. The 5 birds from the positive Wash Bay pool sampled 5 December were all negative, as were all pools from the Nursery. The test results are summarized in Table 1 and shown on a map (Figure 1).

Relative risk of suspect birds contributing to positive swab pools: The probability of there being a suspect bird in a pool was 2.8 times higher if the pool tested positive than if it tested negative.

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Annex 1

Table 1: Epidemiological groups sampled at SANCCOB on 12 Dec- test results and estimated virus prevalence

Epi group	Number of pools tested	Number of positive pools	Prevalence estimate	Prevalence: upper 95% confidence limit	Prevalence: lower 95% confidence limit	No. of birds in Epi group	Estimated no. positive birds
All	85	16	0.044	0.026	0.069	389	17
ICU	12	1 (G126)	0.019	0.001	0.082	52	1
ICU2 (quarantine)	11	2	0.065	0.011	0.189	35	2
Nursery	17	0	0.000	N/A	N/A	86	0
Pen 10 & 11	16	3	0.041	0.010	0.104	78	3
Pen 4	10	2	0.043	0.007	0.127	51	2
Pen 5*	6	6	N/A	N/A	N/A	29	N/A
Pen 8	8	1	0.030	0.002	0.127	33	1
Pen 9	4	1	0.056	0.003	0.226	20	1
Pen 4 & Pen 5	*16*	*8*	*0.120*	*0.060*	*0.228*	*80*	*10*
Pen 8 & Pen 9	*12*	*2*	*0.036*	*0.006*	*0.107*	*58*	*2*

* Calculation does not give accurate estimate when all pools are positive. To provide a very rough estimate: if 5/6 pools had been positive, the prevalence estimate would be 0.3 (0.11; 0.61)

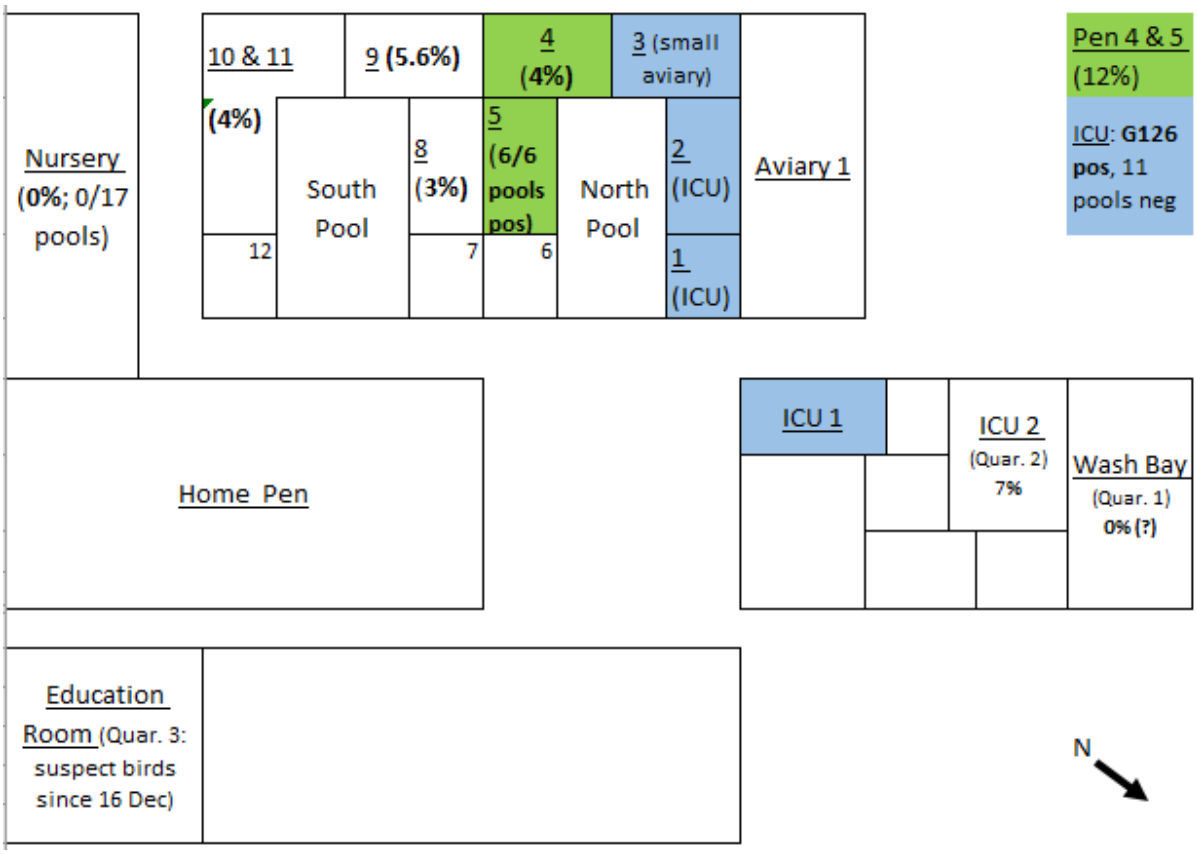


Figure 1: SANCCOB Table View layout and HPAI virus prevalence on 12 Dec

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Annex 1

Discussion

The zero prevalence in the Nursery indicates that complete spatial and functional (different staff and equipment) separation does work to prevent infection.

Virus prevalence in ICU 2, the second quarantined area (after Wash Bay) was slightly higher than most outside pens. However, virus presence in most other areas indicates that it is still widespread and has not been eradicated, and the similar prevalences in pens 4, 8-11 indicate that these areas are not effectively functionally separated. The through-the-fence contact may be a major factor. The reason for the high apparent prevalence in pen 5 is unknown.

Despite the virus being present in most groups, approx. 120 birds have been classified as suspect cases so far, with "cloudy eyes", but only five have been euthanised due to neurological signs and those with cloudy eyes tend to recover normal eyes. Additionally, the initial quarantined group may now be virus-free.

Cloudy eyes is a good indication of virus shedding but removing suspect birds is not stopping virus spread and birds tend to recover from cloudy eyes. Additionally, quarantined birds have compromised welfare: they are indoors and cannot swim properly.

Conclusions

Stop removing suspect cases and rather use presence of cloudy eyes to estimate virus prevalence, and lack thereof to gauge when to re-test.

Positive isolated groups can be moved back outside for welfare reasons, if there is space, as there are also positive birds outside. If there is not space, at least the veranda area outside Wash Bay could now be used.

Keep Nursery, Wash Bay and ICU 1 birds (including the aviaries) separate from others (at SANCCOB) and try working towards getting these birds for release and testing negative. The proposal is to move them to the pens south of the south pool (pens 10-12), leaving the north pens (7-9) empty as a barrier between south pool birds and infected north pool birds.

Authors: Laura and David Roberts (20 December 2022)

Reference

Estimate prevalence from variable pool size and perfect tests, https://epitools.ausvet.com.au/ppvariablepoolsize Williams CJ, Moffitt CM, 2001. A critique of methods of sampling and reporting pathogens in populations of fish. Journal of Aquatic Animal Health 13: 300-309.

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Annex 2

Annex 2.1: African Penguin sampling procedure & sample handling

Action#	Action	Equipment needed	Purpose
FIELD SAMPLING			
1.	Catch & hold penguin	• Gloves • Arm guards (wetsuit sleeves) • Stool/ crate • Stick for lifting birds to check for eggs • Marker spray (Namibia)	Sampling/ body measurements
2.	Measure: Head length Bill length Bill depth	• Vernier calliper (200mm) • (Notebook • Pen)	Sexing Body size vs weight for condition assessment
3.	Cloacal and tracheal/ choanal swabs	• Swabs x 2 • Virus Transport medium • 2ml cryotube • Ice bricks & cooler bag • Side cutters	AIV – PCR (attempt sequencing & isolation if possible). Excess swab fluid to be stored
4.	Feathers: 3 black, 3 white	• Zip lock bag	Sexing (DNA), isotope analysis
5.	Scan for PIT/ microchip	• Microchip scanner/ reader	ID/ transponder project
6.	≤20ml blood	• Needle (21G, 1.5”) (+ 2 spares) • 20 ml Syringe (+ 1 spare) • Gold top serum tubes (PLHG SSTII) 2x 8.5ml • Red top serum tube (CAT) 1 x 4ml • Markers (Artline 725) • Cooler bag & ice brick • Sharps bin (yoghurt tub) • Rubbish bag • Glass slides x 2 • Slide box	• 2ml serum aliquots • Haematocrit & Total serum protein (TSP) • 2 x blood smears • FTA card for PCR- future studies/ haemoparasites • RBCs in ethanol after serum removed from red top tube- backup for FTA card/ haemoparasites

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Annex 2

		• Microhaematocrit/ capillary tubes x 2 • Tube sealer tray/ plasticine • FTA card & Eppendorf tubes (x 1 each) • pencil	
7.	PIT insertion	• PIT tag • Applicator • Alcohol swab	ID/ transponder project
8.	Weigh	• Dog collar/ noose • Hanging scale • Spare scale batteries	Body condition
9.	Ectoparasites (collected opportunistically)	• 70% Ethanol • Eppendorf tubes	Ectoparasite Identification
10.	Faeces (collected opportunistically)	• Eppendorf tubes • 4% Formalin • Small ziplock bag	Identification of GIT parasites
	SAMPLE PROCESSING		
A.	Blood in microhaematocrit tube	• Haematocrit centrifuge • MicroHt reader • Refractometer	PCV TSP
B.	Blood smears	• Fixative • Stain? • Transport boxes	Health Haemoparasites
C.	Serum (±5 x 2ml)	• Centrifuge • Pipettes • 1.8-2ml Cryotubes x 5 per bird • Tube storage boxes • Fridge	AI serology Store for other serology
	GENERAL	• Disinfectant (F10 SC) • Spray bottles • Cloths (kept wet for cleaning) • PPE (overalls & gumboots)	• Power? (battery/ inverter/ solar panels) • Extension cord • Multiplug • Dissection kit (forceps etc) • Receipt book • Pencil sharpener

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Annex 2.2: Biosecurity protocol for field sampling

Objective:

This protocol aims to ensure that there is negligible transfer of pathogens within and between penguin colonies by the survey's team members and their equipment.

Main Principles:

Pathogens can be spread by humans, their clothing and equipment if effective cleaning and disinfection are not implemented. Cleaning involves the removal of gross soiling with bird faeces, other body fluids or secretions, and feathers and mud from a bird colony. Once cleaning is completed, a suitable disinfectant should be applied to inactivate any remaining pathogens. Disinfectant should be mixed to the correct concentration and allowed contact for a sufficient time, to ensure it is effective.

Protocol Details:

All team members will be required to don clean protective clothing before starting work in each colony. It must be possible to remove the protective clothing as soon as work is completed so that the clothing can be placed in a plastic bucket with a lid for storage before washing with disinfectant.

Team members will wear either short rubber boots or plastic sandals, which will allow for thorough cleaning and disinfection each time they enter and leave the colony. Cleaning equipment will include a scrubbing brush.

Team members will wash and disinfect their hands before entering the colony, disinfect after handling of each bird and will wash and disinfect their arms and hands on leaving the colony. Cleaning equipment will include a spray bottle containing disinfectant solution.

All equipment will be cleaned of any gross soiling and disinfected before being used on another bird and will be disinfected after every bird. All equipment will be thoroughly cleaned and disinfected on leaving the colony.

A new, clean hypodermic needle will be used for every bird, for blood sampling.

Disinfectant:

F10 SC Veterinary Disinfectant will be used at a strong concentration of 1:125 (8ml F10 for every litre of water) so that most bacteria, viruses and fungi should be killed after a contact time of 5 minutes. Longer contact time of at least half an hour will be ensured after each day's work, to ensure bacterial spores are killed.

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Annex 3 (African penguin AI vaccine trial)

Annex 3.1- SOP: Blinding procedure

Penguins will be randomly allocated to groups 1 to 4, which will each receive a different vaccine and/or dosing schedule, also randomly allocated.

In order to blind the handlers and the principal investigator, the treatment (vaccine and dosing schedule) applied to each group will not be predetermined or visible at administration.

A random number will be allocated to each treatment (different vaccines and dosing schedules) in an Excel spreadsheet supplied to the monitor and/or SANCCOB veterinarian, and the order of the random numbers will determine the title of the group (1-4). The formula for random numbers in Excel changes the number randomly each time the spreadsheet is opened or edited, so the investigator will have no way of knowing the final numbers and therefore groups. The treatment assigned to each group will be kept by the monitor in the spreadsheet (soft copy and a printed copy) until the data analysis is finalised.

The syringes containing vaccine will be labelled out of sight of the principal investigator and handlers and labelled only with the treatment group number. The label must hide any visible vaccine that appears different from the other vaccine (VLP vaccine is greenish).

All that the blinded people may remember/know is which birds are in the same group but not what was injected to different groups.

Procedure on Day 0:
Vaccine will be drawn up in syringes and placed in four identical containers, two for each vaccine, and labelled with vaccine name. Opaque tape will cover the vaccine in the syringe and provide space for labelling

Vaccine required according to different protocols is supplied in Columns A (day 0) and B (day 32) of the spreadsheet and Random number formulae are supplied in Column C

Step 1: Copy random numbers from column C and paste special as value (s) in column D

Step 2: Sort random numbers in column D from smallest to largest (use the filter function)

Step 3: Insert group name 1-4 in order in column E

Step 4: Move cursor over time in corner of screen if necessary to make both time and date visible, and Print Screen. Paste into MS Word document. OR below…

Step 5: relabel containers securely according to assigned group number and take photograph with syringes visible.

Step 6: Label vaccine syringes only with group number and replace in container

For booster vaccination (day 32):
Again, four sets of syringes will be filled: 6 with VLP vaccine, 6 with inactivated and 12 with control solution (adjuvant / saline) but split between two containers (6 in each). The two groups not receiving vaccine will receive control solution.

The containers and syringes must then be labelled with the group numbers that were randomly assigned on Day 0. This will be done by the monitor.

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Annex 3

Random numbers and groups assigned to birds:

Assign random numbers to each bird

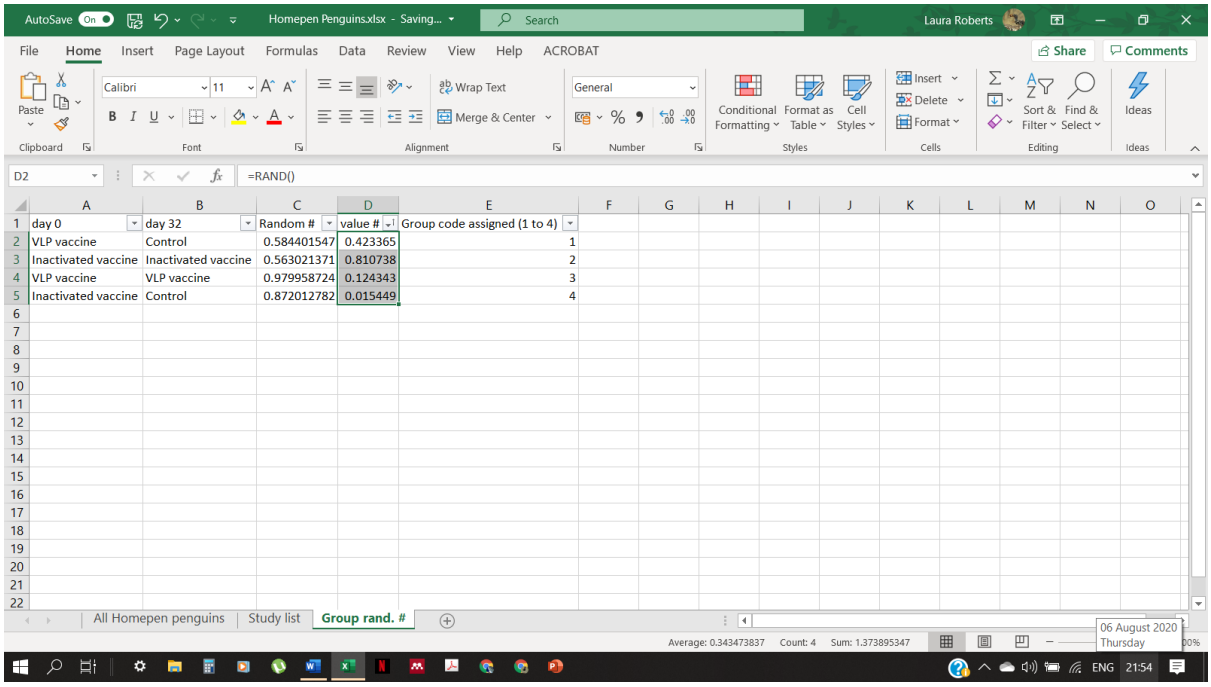
Copy and paste random numbers into next column as values

Filter females, sort by random number and assign, in turn, to groups in order (1-4)

Filter males, “, continue assigning groups wherever the females left off

Remove filter from sex.

Screenshot of random numbers assigned to penguins:



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Annex 3

Annex 3.2- SOP: Clinical examination, blood sampling, vaccination and record-keeping

Record results of weighing, clinical exam and vaccination and bleeding procedure on Form 1

Labelling
Serum tubes labelled: bird number, date, "L Roberts"
Blood slides labelled: Bird number, bird name, date

A. Handling of penguin:

The veterinarian performing the sampling and vaccination will be assisted by an experienced penguin handler (a SANCCOB staff member), who will restrain the bird according to documented SANCCOB protocols

1. Pick up bird
2. Place in crate on scale
3. Check name on flipper band, flipper band number & record body mass
4. Scan microchip & check against Form 1
5. Vet performs quick clinical examination to measure heart rate and assess breathing & chest sounds and mucous membrane colour, mouth, eyes, feet: record on form 1
6. For vaccination into the pectoral muscle, the penguin will be held upright with one of the handler's hands controlling the head and the other supporting the bird's weight and holding the feet. The flippers are controlled by pinning them between the handler's arms and body.
7. Vaccination will consist of injection of 0.25ml vaccine intramuscularly in the pectoral muscle, using a new, sharp and clean 1/2", 26 G needle. The plunger will be withdrawn after insertion of the needle, before injection, to ensure no blood vessel is entered. Mark off on Form 1 once done. Place syringe & needles in VACCINE sharps container
8. Jugular sampling: the handler will be seated with the penguin held between the handler's legs, facing away from the handler. The head of the bird is gently but firmly held behind the eyes and the neck extended and stretched laterally over the handler's right knee. The handler covers the bird's eye to reduce stress.

   The vet will occlude the left jugular vein with his or her left thumb and insert the needle into the raised jugular vein. After blood is collected into the syringe, the handler will put moderate pressure onto the venipuncture site and return the head to upright position. The right vein will only attempted if unsuccessful after 2 needle insertions on the left side.

   Mark off on Form 1 once done.
9. The bird will be held still for at least 2 minutes, by the handler, with pressure on the puncture site in the jugular vein after the blood has been withdrawn, to prevent haemorrhage.
10. Penguin placed in pen with others already processed and monitored every 15 minutes for an hour for signs of allergic reaction to vaccine and for haemorrhage from jugular.

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B. Blood Sample handling:

1. Needle is removed from syringe and blood transferred gently to 5ml gold-topped serum separator tube (SST). Needle and syringe disposed of in SANCCOB sharps/ biohazard bins.
2. Sealed serum tubes should be gently inverted 180º and back 5-6 times
3. Blood from hub of needle/ neck of syringe used to draw up blood in 2 microhaematocrit tubes
4. Blood from MicroHt tube used to make a blood smear
5. MicroHt tubes plugged and placed on press-stick on Form 2 (blood smear and Ht/PCV sheet)

C. Serum tube processing:

1. Keep tubes cool and out of sunlight
2. Centrifuge: 3000 g for 15 minutes
3. Refrigerate and transport to laboratory on ice.

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Annex 3.3- SOP: Adverse reactions to vaccination or blood sampling

Allergic reactions:

Any clinical signs or adverse effects will be recorded and managed and each bird will receive a clinical evaluation before and after each sampling.

Little to no reaction to the vaccine is expected, but the birds will be monitored for an hour after vaccination so that any allergic reaction can be detected and treated.

Any bird showing signs of respiratory distress or adverse reaction to the vaccine will be placed in an oxygen box or oxygen will be supplied via endotracheal tube under sedation, if necessary. Antihistamine will be administered and adrenaline and intravenous fluids if necessary. If bronchoconstriction is suspected due to difficulty breathing and wheezing, salbutamol can be administered via asthma pump or nebulizer.

Blood sampling

To reduce the chance of bleeding from the sample collection site the penguins will be held for two minutes with pressure on the site after the blood collection is complete.

In the event of excessive bleeding pressure will be maintained for a further five minutes and if necessary intravenous fluids will be given after inserting an intravenous catheter under general anaesthesia.

Equipment & drugs required in sampling area/ pen

3ml adrenaline (1ml/kg) in syringe with 26G ½" needle

Spare adrenalin vials

Salbutamol inhaler

Antihistamine

Oxygen box, canister and regulator

Equipment ready in clinic:

Sedative

ET tube & ambubag

i/v set: fluids, drip set, i/v catheter, Elastoplast etc

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Annex 3.4- Procedures and monitoring to ensure optimal bird welfare (by D Roberts)

Procedures to catch birds in home pen

Prevent the home pen penguins from entering the pool by setting up barriers around the pool. Areas of home pen and entrances to nest boxes can be blocked off as well to reduce the distance that penguins can run to escape.

Two to four trained people can then gently herd penguins into pen areas from where they can be caught. Penguins that are easy to catch can be picked up and placed in boxes when it is convenient to do so. Penguins in nest boxes can be removed from there.

All catching should be done with a minimum of noise and with no rapid movements so as to minimise the disturbance of the other home pen birds.

Penguins that are part of the study will be placed into SANCCOB transport boxes to be moved to the sample collection area. Those that are not part of the study can be placed into a separate quiet area of home pen.

Any study birds sitting on eggs can be left alone though barriers will be placed around the nest box, and they will be fetched later, one member of the pair at a time.

Reducing stress and improving welfare during catching

Plan the catching process before hand

Experienced staff only to herd and catch birds

Reduce the penguin escape distance to a minimum by using barriers and herding the penguins into a smaller area before catching begins.

Work purposefully without rushing or making a noise and reduce sudden movements to a minimum.

Work methodically from one area to the next.

Have all boxes and barriers in place before starting

If possible, attempt to have all birds caught before the day starts in the rehab facility to reduce additional external noise

This must be done during the cool part of the day in summer.

Signs of stress in home pen during capture

A good indication of the level of stress caused by catching the home pen birds can be gauged by observing the behaviour of both the penguins and the other birds during the catching process. There will always be some distress caused by a disruption in the normal routines and more if many birds are being caught.

Signs of excessive stress will include penguins running for long distances (especially if tripping, falling over while running or colliding with obstacles is seen), lots of penguins running together in a group or stampede, excessive vocalisation, rapid breathing or aggression shown to other penguins such as biting or flipper slapping. If the home pen flying birds get distressed they will start to fly repeatedly from one side of the pen to the other. If any of these signs are seen, the capture attempt should be halted until the birds calm down and a better capture method attempted.

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The two swift terns in Home pen are extremely sensitive to stress and need to be monitored closely. Vocalisation and dropped wings are a clear indication that they are stressed.

Locations suitable for sampling

The sampling area needs enough space to house the penguins, the research team, and their equipment and be out of the way of normal SANCCOB activities.

Suitable areas include the old nursery building, the wash bay and pens number 9, 10, and 11 if they are unused.

5 pens to be set up, if there is space.

Available sites may change during chick season

Keeping birds before and after sample collection

Before and after sampling, the penguins should be kept in pens, preferably away from disturbance by other activities. Different pens should be set up for birds before and after sampling. Good ventilation and protection from excessive disturbance, heat and cold must be ensured. Birds can be placed in pens in suitable social groups.

Reducing stress around and during sample collection

Human noise and sudden movements must be kept to a minimum. Casual conversations should be held away from the sampling area. Moving and dragging of items at the washing station outside Nursery should be avoided.

Each penguin must be handled for as short a time as possible, therefore all equipment and team members must be ready for the procedures before handling starts.

The penguins should only be caught and handled by trained SANCCOB personnel, according to the SANCCOB bird handling SOPs and the project guidelines for blood collection.

For jugular blood collection, it is more efficient and less stressful for the penguins if they are held and handled purposefully and firmly. Excessively gentle handling will lead to more wriggling and escape attempts by the penguins. This could lead to reduced effectiveness of bleeding, possibly more bleeding attempts and more handling time. Cover eyes with hand/ towel when possible.

Signs of stress must be noted and, if they are excessive, the handling should be stopped. It is important that the bleeder listen to the holder if they are worried about positioning of the bird as well as the stress of the bird,

Signs of stress during handling and sample collection

Any handling and restraint of penguins will cause stress and so it is essential to reduce this stress to a minimum.

Penguins will show that they do not want to be handled by resisting capture, struggling and attempting to bite and flipper slap. This is normal behaviour for a penguin when it is restrained or handled. Increased stress will lead to increased struggling and escape attempts and increased respiratory rate. Excessive stress can lead to panic, panting, vocalisation, weakness and exhaustion.

If any penguin shows signs of panting or exhaustion all handling must be stopped immediately and appropriate veterinary care given.

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Welfare monitoring after sample collection

All the penguins will be placed into a pen for monitoring for at least one hour after blood sampling. During this time, respiratory rate, eye patch colour and activity will be monitored for any abnormalities and recorded every 15 minutes.

Return to home pen

After the hour of monitoring, the birds can be returned to home pen.

Welfare monitoring after sample collection

Throughout the project time frame a special home pen feeding list will be used to record the welfare of the home pen birds twice a day during feeding. The aim is to determine if the birds are unusually stressed because of the project but they must also be monitored so that any sign of illness and specifically, infection/ abscess at the injection site. Each bird's appetite, physical appearance and behaviour should be recorded as either normal or abnormal for that individual. If anything is abnormal, notes should be taken and discussed with the vet on duty. This form must be filled in by the responsible rehabber and filed every day. Any abnormalities and subsequent decisions must be recorded on the WRMD computer system.

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No.	Name	Band #	Microchip	Mass (kg)	Chest	MM	Feet	Eyes	Mouth	HR	RR	Group	Vaccine?	Blood?
1	Alberta	SB 014	982 000 365 451 657									3		
2	Beak-Boy	SB 027	982 000 365 560 309									2		
3	Cuddles	SB 289	982 000 365 452 232									1		
4	Funny Flippers	SB 025	800 158 1510									3		
5	Hansel	SB 006	800 152 8553									2		
6	Heiffer	SB 284	118 001 180 061									2		
7	Juniper	SB 016	982 000 365 451 447									3		
8	Kia	SB 300	982 000 365 452 096									1		
9	Meister	SB 265	982 000 365 857 141									1		
10	Morgan/ Smudge	SB 262	945 000 001 574 100									4		
11	Nona	SB 272	982 000 365 860 659									2		
12	Norbert	SB 281	989 001 004 364 560									3		
13	Pamela	SB 271	100 437 6754									3		
14	Peepp	SB 282	982 000 365 451 537									4		
15	Princess	SB 019	118 001 530 457									1		
16	Que	SB 015	945 000 001 573 230									1		
17	Shadow	SB 022	800 158 1810									2		
18	Skipper	SB 277	096 400 000 617 2822									4		
19	Steve	SB 007	100 437 7473									2		
20	Stubby	SB 026	982 000 365 857 045									1		
21	Sweet Pea	SB 012	000 365 556 0326									4		
22	Syd	SB 047	982 000 365 560 406									4		
23	Sylvester	SB 046	982 000 365 811 905									4		
24	Winston	SB 031	090 011 800 158 2316									3		

No.= study number, Chest/MM (mucous membranes)/ feet/ eyes/ mouth: normal? HR= heart rate (beats per minute), RR= respiratory rate (breaths per minute), group= treatment

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African Penguin AI Vaccine Trial

Blood Smear, TSP, PCV Results

Date Sampled_____ Date Read_____

Reader _____

	Bird name	Blood smear Result	PCV	Buffy coat	TSP	On Card
1	Alberta					
2	Beak-Boy					
3	Cuddles					
4	Funny Flippers					
5	Hansel					
6	Heiffer					
7	Juniper					
8	Kia					
9	Meister					
10	Morgan/ Smudge					
11	Nona					
12	Norbert					
13	Pamela					
14	Peepp					
15	Princess					
16	Que					
17	Shadow					
18	Skipper					
19	Steve					
20	Stubby					
21	Sweet Pea					
22	Syd					
23	Sylvester					
24	Winston					

If a bird has a value of 3+ please give the cell count per 50X high power field. EG H3+ (20/hf)

Normal: Packed Cell Volume (PCV): 38-48%,

Buffy Coat (BC): 0-2mm,

Total Protein (TP): 4-6 g/dl

If PCV is below 30 or above 48, or TP is below 3: Inform the vet and re-bleed to confirm the result

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African penguin AI vaccine trial - welfare monitoring after procedure

Names of people

Date: _____

Vet		
Handler		
Monitor		

Penguin Name												
Time (every 15 mins)												
Respiratory rate (Breaths / minute)												
Activity												
Eye patch colour												
Signs of swelling												
Respiratory quality												
Demeanour, posture & appearance (0-3)												
Locomotion (0-3)												
Temperament / Personality (0-3)												
Initials of monitor												
Other observations and interventions	**Vaccination/ bleeding time:**						**Vaccination/ bleeding time:**					

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Home Pen Penguin Feeding: Tick if ate normally & comment on any abnormalities in appetite, behaviour or physical appearance (MOBIFLEX). Week starting Monday, ___ _____ 2020

		Monday			Tuesday			Wednesday			Thursday			Friday			Saturday			Sunday		
FEEDER's INITIALS		am		pm	am		pm	am		pm	am		pm	am		pm	am		pm	am		pm
Name	SB#	1	2	Comments	1	2	Comments	1	2	Comments	1	2	Comments	1	2	Comments	1	2	Comments	1	2	Comments
ALBERTA	014																					
ALFIE																						
BEAK-BOY	027																					
CEDRIC	050																					
CUDDLES	289																					
EDGAR	020																					
FLIPPER	280																					
FLO	023																					
FUNNY FLIPPERS	025																					
HANSEL	006																					
HEIFFER	284																					
JAEGER	018																					
JUNIPER	016																					
KARSTEN	005																					
KIA	300																					
LAMBERT	013																					
MEISTER	265																					
MILO	011																					
MORGAN SM.	278																					
MOSELEYI																						

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YUNIBESITHI YA PRETORIA

Home Pen Penguin Feeding: Tick if ate normally & comment on any abnormalities in appetite, behaviour or physical appearance. Week starting Monday, ___ _____ 2020

		Monday			Tuesday			Wednesday			Thursday			Friday			Saturday			Sunday		
Name	SB#	1	2	Comments	1	2	Comments	1	2	Comments	1	2	Comments	1	2	Comments	1	2	Comments	1	2	Comments
NESSIE	302																					
NONA	272																					
NORBERT	281																					
NOTY	266																					
PAMELA	271																					
PEEPP	282																					
PETA	267																					
PRINCESS	019																					
QUE	015																					
ROCKY																						
SHADOW	022																					
SKIPPER	277																					
STEVE	007																					
STRING	017																					
STUBBY	026																					
SWEET PEA	012																					
SYD	047																					
SYLVESTER	046																					
WINSTON	031																					

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Annex 4.1

South African seabird disease outbreak preparedness plan

Draft 4: May 2024

Contributors: L Roberts, A Kock, M Makoala, L Nicholson, L Christian, D Zimmerman, D Roberts, P de Villiers

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Annex 4.1

Definitions

Clinical sign – a behavioural or physical change from normal, expressed by an individual when suffering from a disease (similar to a disease symptom, but symptoms are experienced and described by human patients, so do not apply to animals)

Controlled disease - any animal disease in respect of which any general or particular control measure has been prescribed in the Animal Diseases Act (Act 35 of 1984) Regulations, Table 2, and any animal disease which is not indigenous to South Africa

Disease – any impairment of the normal structural or physiological state of a living organism resulting from its physiological response to a hazard **epidemic**

Epidemic (epizootic) – an increase in the prevalence and/or incidence of a disease, above what is expected, in a defined geographic area

Fomite- inanimate objects that can carry and spread pathogens

Incidence – the number of new health events (infection, disease etc.) experienced by a given population over a specific period.

Outbreak- an increase in incidence of a disease, when cases are in excess of what is considered normal for the location or season, in a limited geographic area (smaller than for an epidemic).

Prevalence – the proportion of the host population that is affected (e.g. with infection, disease or antibody presence), often expressed as a percentage. A measure of how widespread an infection, disease or exposure to an infectious agent is at a point in time.

Seabird (= marine bird) - coastal or pelagic bird species that is dependent on the ocean for survival, beyond the surf zone (Audubon California)

Zoonosis: a disease that can be transmitted from animals to humans

Abbreviations

AHT- Animal Health Technician
DALRRD- Department of Agriculture, Land Reform and Rural Development
DEADP- Department of Environmental Affairs and Development Planning
DFFE- Department of Forestry, Fisheries and the Environment
HPAI- High pathogenicity avian influenza
MPA - Marine Protected Area
NEMA- National Environmental Management Act (Act No. 107 of 1998)
PA- Protected Area
SANCCOB- South African Foundation for the Conservation of Coastal Birds
SANParks- South African National Parks
TOPMS- Threatened or Protected Marine Species

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Chapter 1 – Introduction and background

1.1. Scope and objectives

Mass mortality in seabirds, due to high pathogenicity avian influenza (HPAI), demonstrated the need for better preparedness for such incidents. Role-players and activities required to respond to disease outbreaks were previously not familiar to conservation authorities and field staff, which hampered response efforts.

This document aims to supply information on role-players and their responsibilities, as well as activities that may be required to respond to disease outbreaks causing mass mortality in seabirds in South Africa. Although the characteristics of individual diseases may require slightly different guidelines, general principles and higher-level response will be common to all diseases and are described here. Specific requirements for prioritised disease will be provided separately, as individual protocols. Conditions at individual sites will also differ, so specific guidelines cannot be supplied for every situation, and this document aims to guide the compilation of site-specific contingency plans (Annex 4.3), which can be disease-specific, based on the protocol, or may provide for different diseases.

1.2. Categories of diseases and basic principles

Diseases most likely to result in mass mortalities include:

- Infectious diseases
  - Diseases caused by microorganisms including viruses, bacteria, fungi and protozoa, and macro parasites including arthropods (ticks, fleas, lice) and worms.
  - Contagious: caused by a pathogen acquired directly from an infected animal or indirectly via inanimate objects (fomites).
  - Vector-borne: a disease is transmitted from one animal to another by a living agent (vector), such as an insect, mite or tick.
- Toxic diseases- diseases caused by exposure to a substance above levels at which an animal can process or eliminate it, leading to interference with biological processes or physical or physiological damage.
- Nutritional diseases- diseases caused by deficiencies of different nutrients required for physiological functions.

The epidemiological triangle: The manifestation of a disease depends not only on the presence of a **pathogen and the pathogen's characteristics,** but also on characteristics of the **animal** and **environmental conditions**. These three factors form the corners of the epidemiological triangle. Animal characteristics include the susceptibility of the animal to the pathogen, determined by species, sex, age, and immune status (linked to stress and nutrition). Relevant environmental characteristics are those that may favour transmission or survival of the pathogen or susceptibility of the animal. Extreme environmental factors may also increase stress levels, flight or swimming distance to a food source, ocean conditions such as sea temperature swell size, or predation rates. Favourable transmission conditions can include a large number of animals in close proximity to each other, for example a breeding colony or roosting site.

1.3. Disease management principles and objectives

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The aim of disease management is to effectively disrupt the epidemiological triangle. This requires an understanding of the ecosystem and susceptible species and associated stressors, and identifying the disease, high risk sites, and risk management methodologies. Options include:

- Destroy the pathogen
  - In the environment (e.g. disinfectants)
  - In the animal
    - Target the pathogen in the animal (e.g. antibiotics/ antivirals)
    - Destroy and dispose of the animal (e.g. remove sick birds and infected carcasses)
- Destroy the vector/ intermediate host (e.g. kill malaria-carrying mosquitoes)
- Prevent infection in the animal
  - Re-inforce/ induce immunity
    - Vaccination- teach the animal's immune system how to recognise and fight the disease
    - Support immune system (decrease stress, improve nutrition)
  - Prevent indirect contact with the pathogen or toxin by ensuring that they do not have access to contaminated objects.
  - Prevent aggregation/ congregation and high density situations where more animals are exposed and the disease can spread quickly

1.4. Historical disease outbreaks in South African seabirds

- High pathogenicity avian influenza (HPAI): More than 1300 Common Terns birds were found dead between Lambert's Bay and Gqeberha in 1961 and HPAI was eventually identified as the cause. HPAI (H5N8) killed more than 5400 Swift Terns and 100 African penguins in 2018 and H5N1 led to the deaths of at least 24 000 Cape Cormorants and 330 African Penguins in 2021-2022.
- Avian cholera caused by the bacterium *Pasteurella multocida*: large epidemics have occurred worldwide and six outbreaks had been reported in nine species of South African seabirds by 2007, including Kelp Gulls, Cape Gannets, Swift Terns, Hartlaub's Gulls, White-breasted Cormorants and Cape Cormorants. Cape Cormorants were most affected and most were on Dyer Island where an outbreak occurred every year between 2002 and 2005 (Waller and Underhill, 2007).
- Botulism frequently affects Kelp Gulls, sometimes in large numbers but outbreaks also occur in waterfowl.

1.5. Coastal seabird conservation (A. Kock)

In addition to disease, South Africa's seabirds face various natural and anthropogenic threats. These threats can predispose seabirds to diseases, and affect the outcome, e.g. stressed or starving birds may be more easily infected and be more likely to die than birds that were healthy before infection. The effect of a disease outbreak on a species or population of birds that is struggling to cope with other threats is more likely to cause a significant and long-lasting effect on the population.

Major threats include:

1. Reduced prey availability: Many seabirds rely on fish (e.g. sardine and anchovy) and other marine organisms (e.g. rock lobster and mussels) as their primary food source. Overfishing and the depletion of fish stocks can reduce food availability for seabirds, negatively impacting their survival and populations. In addition to overfishing, prey numbers are

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affected by pollution, climate change and any other factors which contribute to biodiversity loss.

2. Habitat destruction: Seabirds rely on specific breeding, nesting, and foraging habitats. Destruction or degradation of these habitats through overfishing, urban development, oil spills, historic guano harvesting and invasive species can significantly negatively impact seabird populations.
3. Pollution: Oil spills, plastic debris, sewage and other sources can significantly impact seabirds, causing mass mortalities, reduced breeding success, increased mortality, and reduced food availability.
4. Climate change: Changes in sea surface temperatures and ocean currents can affect the distribution and abundance of prey species, affecting seabirds' foraging effort and success and ultimately breeding success and survival. Changes in climate and prey-availability can lead to unseasonal breeding. Extreme weather events can lead to high chick and egg mortality and poor breeding success.
5. Predators or invasive species: Predators such as Cape fur seals, Kelp Gulls, caracals, or leopards and invasive species such as rats, cats, and mice can devastate seabird populations by preying on eggs and chicks.
6. Human disturbance: Seabirds are sensitive to human disturbance, which can disrupt their breeding and nesting behaviour and their ability to commute and forage effectively (shipping disturbance), leading to reduced breeding success and increased mortality.
7. Disease: Diseases such as avian cholera and avian influenza have led to mass mortality in several species of South African seabirds. Birds that nest in dense colonies are particularly at risk.

Marine bird management plans (e.g., African Penguin Biodiversity Management Plan) and management actions included in Protected Area (PA) Management Plans (often marine bird breeding colonies exist in PAs) list specific management actions in an attempt to mitigate the above challenges and stressors, e.g. artificial nest boxes and breeding platforms, predator control, restrictions to fishing around islands, PA expansion, etc. These systems and processes need to be effectively monitored and continually evaluated to ensure they are achieving the desired outcomes. Interventions, including invasive monitoring techniques, may contribute to an increase in bird stress levels themselves and may also contribute to the spread of disease if not carried out under clear guidelines and SOPs.

Chapter 2 – Constitutional, legislative and policy mandates

2.1. South African Legislation and policy

2.1.1.Environmental management

Environmental management is a joint mandate in South Africa, between the different spheres of government: national, provincial and local. One of the environmental rights that the Constitution affords everyone is the right to a protected environment. This means that the Constitution places a mandate on the government to protect the environment through reasonable legislative means and other measures. In order to realise this constitutional mandate, the government has revised or promulgated various laws. These are:

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- National Environmental Management: Protected Areas Act (No 57 of 2003; NEM:PAA). All activities related to prevention, mitigation, and response need to comply with NEMPAA to ensure overall ecosystem and habitat integrity. This Act provides for the protection and conservation of ecologically viable areas, as well as the management, co-operative governance, public participation and matters relating to protected areas. Seabird colonies fall within the protected area network as classified under Section 9 of the Act. The protected areas' purpose is to preserve the ecological integrity and protect threatened or rare species, their ecosystems and habitats.

- National Environmental Management: Biodiversity Act (No 10 of 2004; NEM:BA) including Threatened or Protected Species regulations. This provides for the protection of species and ecosystems in need of protection. NEM:BA has various tools that contribute towards the conservation of seabirds, and these include:
  - Policy on management on Seals, Seabirds and Shorebirds (2007) (Marine Living Resources Act, 18 of 1998). Although no specific section has been written on diseases, the policy does indicate that seabirds are not released back into their normal breeding sites until they are certified to be disease-free by a qualified veterinarian, because of the risks of disease transmission.
  - The Threatened or Protected Marine Species (TOPMS) regulations (2017) aim to regulate the permit system as it applies to Chapter 7 of NEM:BA, involving listed threatened or protected marine species. The Regulations further provide guidance for listed threatened or protected marine species that are kept in captive breeding facilities, rehabilitation facilities, sanctuaries and so forth. They aim to either prohibit or regulate the manner in which specific restricted activities may be carried out.
  - Draft Biodiversity Management Plan for African penguin (2019)- Gazetted version 2013. The review of the African Penguin Biodiversity Management Plan was gazetted in 2022 for public comments following a further decline in the African penguin population. The draft BMP outlines diseases as one of the major threats that could contribute to the decline of penguins, through the death of adult birds or by reducing breeding success. The BMP further stipulates that the efficacy of the implementation of the African Penguin Disease Surveillance and Diagnosis Programme needs to be assessed, and that all colonies have Disease Contingency Plans with associated accessible resources for rapid management action.
  - The Draft norms and standards document relating to the management of seabirds in captivity, gazetted Oct 2019, identifies diseases as one of the major causes of seabirds' mortality and encourages captive and rehabilitation facilities to conduct post-mortem examinations to establish the cause of death. The Norms and Standards further provide standards for disease control. Furthermore, the Norms and Standards also encourage facilities to have Contingency Plans in place to deal with events that occur outside the range of normal operations and that may adversely affect the centre's ability to operate normally.
- National Environmental Management: Integrated Coastal Management Act, 24 of 2008: promotes conservation of the coastal environment and those responsible and controls incineration and dumping at sea.
- The National Environmental Management: Waste Act, 59 of 2008
- The National Environmental Management: Air Quality Act, 39 of 2004

2.1.2.Animal diseases and their treatment and animal welfare

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- Veterinary and Para-Veterinary Professions Act (Act No.19 of 1982) provides for control over the practising of veterinary and para-veterinary professionals (e.g. veterinary nurses and animal health technicians) and for services which can be rendered only by a registered veterinary professional. The Rules Relating to the Practising of Veterinary Professions define these as, among others, diagnosis of and providing advice on preventing disease in an animal and any surgical procedure on an animal. Invasive sampling techniques are included here.
- Animal Diseases Act, 35 of 1984 (mandate of the national Director: Animal Health and provincial Veterinary Services) E.g.:
  - Section 11- Duties of owners and managers regarding the health of animals
  - Section 12- Reporting of incidence of controlled animal diseases [9(2)*(h)*, 11(1)*(b)*(ii)]. The list of Controlled Diseases is available on the DALRRD website but the main diseases of importance in birds are avian influenza, Newcastle disease, Salmonella and Psittacosis (*Chlamydia psittaci*).
  - Section 20- Limitations on investigations, experiments and research with, and manufacture and evaluation of, animal products

  Owners of animals should protect their animals against disease, prevent their animals from being a source of disease for other animals and report any suspected controlled disease.
- Animals Protection Act, 71 of 1962- protects animals against cruelty and abuse, implemented by the National Society for the Prevention of Cruelty to Animals (NSPCA)

2.1.3.Other

- Disaster Management Act (2002) amended 2015. A national Disaster Management Centre, under the Department of Cooperative Governance, provides guidance for provincial disaster Centres, which in turn guide centres in district municipalities.
- Municipal Systems Act, 32 of 2000
- South African Maritime Safety Authority (SAMSA) Act (Act 5 of 1998)

2.2. International agreements and conventions

- Agreement on the Conservation of African-Eurasian Migratory Waterbirds (AEWA) https://www.unep-aewa.org/
  - AEWA International Multi-species Action Plan for the Conservation of Benguela Current Upwelling System Coastal Seabirds (2015)
- Convention on the Conservation of Migratory Species of Wild Animals (CMS) https://www.cms.int/
- Ramsar Convention on Wetlands https://www.ramsar.org/
- Convention on Biological Diversity https://www.cbd.int/

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Chapter 3 – Integrated Institutional Capacity

Many seabird colonies are located in an existing protected area. There are Management Authorities (i.e. SANParks, CapeNature, Robben Island Museum) managing those respective areas in nature reserves or national parks. Colonies falling outside the ambit of a protected area are mainly managed by the respective municipalities. These Management Authorities have been issued with the respective permit from DFFE to manage marine species under the Threatened or Protected Marine Species Regulations (TOPMS Regulations).

Local Municipalities usually manage public beaches (an exception is Langebaan beaches in the Marine Protected Area (MPA) that are managed by SANParks). Therefore, sick or dead seabirds found on public beaches outside reserves/colonies may need to be managed by them. Should local municipalities require further assistance in terms of resources, then matters are escalated to the respective provincial entities responsible for conservation and then to a national department.

3.1. National Government

Department of Forestry, Fisheries and the Environment

DFFE is a regulatory authority responsible for environmental management, conservation and protection. The Branch: Oceans and Coasts provides an oversight role and ensures the regulation and management of marine biodiversity, while supporting other relevant stakeholders.

- Branch: Oceans & Coasts
  - Directorate: Oceans Conservation Strategies
  - Directorate: Biodiversity and Coastal Research
  - Directorate: Monitoring (This Directorate consists of Coastal & Marine Monitors that each conduct regular patrols along long stretches of the South African coast
- Branch: Chemicals and Waste
- South African National Parks (SANParks)
  - Respective park management (Namaqua NP; West Coast NP; Table Mountain NP; Agulhas NP; Wilderness/Tsitsikamma; Addo Elephant NP). Park Managers supervise Area Managers, who supervise colony managers.
  - Conservation Services Division
    - Scientific Services – there are three marine ecologists in SANParks who provide support services to park management
    - Veterinary Wildlife Services - veterinary support for park management, assist with disease management and training

Department of Agriculture, Land Reform and Rural Development

- Branch: Agriculture Production, Health and Food Safety
  - Chief Directorate: Animal Production & Health (The Chief Director is also the Chief Veterinary Officer for South Africa)
    - Directorate: Animal Health (DAH): Whose mandate is related to the promotion, prevention and control of animal diseases, reducing sanitary risks in the import and export of animals and animal products and rendering epidemiological services for early warning and monitoring of animal diseases. Policy approved by the national DAH guides

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the actions taken by provincial veterinary services. The DAH reports on animal diseases to the World Organisation for Animal Health (WOAH, previously OIE).

Department of Cooperative Governance

- National Disaster Management Centre

3.2. Provincial Government

Western Cape (WC)

- Department of Environmental Affairs and Development Planning
  - CapeNature: Responsible for conservation areas and wildlife in the province.
  - Directorate: Waste Management
- Department of Agriculture
  - Programme: Veterinary Service
    - Sub-programme: Animal Health is mandated to enforce the Animal Diseases Act. They can advise on options available to limit the effects of a controlled disease and, where personnel are available, assist with the sampling of animals suspected to be infected with a controlled disease. The provincial Director: Animal Health is based at the Department of Agriculture (DOA) headquarters on Elsenburg farm, near Stellenbosch, along with two State Vet: Epidemiologists who are responsible for overseeing disease surveillance and reporting across the province. The province is divided into eight State Vet Areas, which are each the responsibility of a State Veterinarian, with 3 to 5 Animal Health Technicians (AHT) per state vet area, each responsible for their own smaller area. The areas and contact details of the responsible state vets are available at https://www.elsenburg.com/veterinary-services/animal-health-and-disease-control/
    - Sub-programme: Veterinary Diagnostic Services (Western Cape Provincial Laboratory) https://www.elsenburg.com/veterinary-services/veterinary-laboratory-services/ is based at the laboratory in Stellenbosch and offers testing for various animal disease agents and can assist with some toxin testing. They also offer an animal post-mortem/ necropsy service. Prices are available on the website and there is limited free testing for cases that Animal Health considers of importance in provincial disease surveillance.
- Department of Local Government
  - Directorate Disaster Management and Fire & Rescue Services- disaster prevention, risk reduction and mitigation through disaster preparedness, response and recovery and rehabilitation.
- Department of Health- needs to be kept informed of any potential zoonotic pathogens, to plan their detection and the management of potential human infections (communication, testing, treatment etc.)
  - Deputy Director: Communicable Disease Control

Eastern Cape

- Department of Economic Development, Environmental Affairs and Tourism (DEDEAT)
  - Eastern Cape Parks and Tourism Agency (ECPTA)

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- Department of Rural Development and Agrarian Reform (DRDAR)
  - Programme: Veterinary Services (Veterinary laboratories Mthatha, Grahamstown (Makhanda) (not SAVC registered)

KwaZulu Natal

- Department of Economic Development, Tourism & Environmental Affairs (DEDTEA)
  - Chief Directorate: Environmental Management
    - Ezemvelo KZN Wildlife
- Department of Agriculture and Rural Development (DARD)
  - Branch: Agricultural Development Services- Vet Services (Allerton Provincial Veterinary laboratory https://www.kzndard.gov.za/allerton-laboratory)

Northern Cape

- Department of Agriculture, Environmental Affairs, Rural Development and Land Reform
  - Programme 4: Veterinary Services (NC Provincial Veterinary Laboratory, Kimberley)
  - Programme 8: Environmental Affairs

3.3. Other

Robben Island Museum is responsible for birds on Robben Island and employs an Environmental Manager.

Rehabilitation Centres

- SANCCOB- centres in Table View, Western Cape (serving Cape Town, West Coast) and in Gqeberha, Eastern Cape. Additional Seabird Rangers are deployed on Robben, Dassen and Bird (Algoa Bay) Islands and at Stony Point and in Simon's Town. They assist the managing authorities with daily monitoring.
- African Penguin and Seabird Sanctuary (APSS), Dyer Island Conservation Trust (DICT)- Gansbaai/ south coast (Western Cape).
- SAPREC- Mossel Bay
- Ushaka Seaworld (KZN coast- penguins)
- CROW (Between Umkomaas to northern KZN regions -Sea birds except penguins)
- SCAR (Umkomaas to southern KZN regions - Seabirds excluding penguins)

Contact details are provided in Annex 4.1.1.

Aquaria

- Two Oceans Aquarium (Cape Town)- daily patrols at V&A Waterfront and Cape Town harbour- Marine Wildlife Management Program Coordinator
- UShaka Marine World (Durban)- linked with KZN Strandings Network
- Bayworld (Gqeberha)/ East London Museum: marine mammal and turtle strandings- monthly patrols in two areas, east and west of Gqeberha, in collaboration with Nelson Mandela Metropolitan University. To the west, cover 20km coast in St Francis Bay (the bay itself), and to the east, 40 km in Algoa Bay. Agents also assist with monitoring and reporting.

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Academia

- University of Cape Town
  - Percy Fitzpatrick Institute of African Ornithology
- Nelson Mandela Bay University
  - Coastal and Marine Research Institute
- University of Pretoria
  - Faculty of Veterinary Science

BirdLife South Africa (https://www.birdlife.org.za/) is a non-profit bird conservation organisation, and is the country partner of BirdLife International. Birdlife SA runs research and conservation projects and promotes birding and general appreciation of the environment.

National Sea Rescue Institute (NSRI) – is sometimes able to assist with transport to and from islands https://www.nsri.org.za/rescue/base-finder

Stranding Networks- linked in via Provincial Environmental management agency

West Coast
Greater Overberg
Mossel Bay (SMART)
Plettenberg Bay
KZN Marine Stranding Network

Estuary Advisory Fora: these form good communication platforms and exist at all larger estuaries along the Western Cape coastline. Often marine birds roost in or adjacent to these relatively protected ecosystems, e.g. Berg Estuary and Heuningnes Estuary

Other NGOs

World Wide Fund for Wildlife (WWF)
Endangered Wildlife Trust (EWT)
Wild Oceans (KZN)
Blouberg conservation authority
SeaSearch
Nature's Valley Trust

South African Maritime Safety Authority (SAMSA) - SAMSA Act 5 of 1998

South African Veterinary Council (SAVC) and **Association (SAVA)** represent veterinarians and para-veterinary professionals. The SAVC is the veterinary statutory body, with which all veterinary professionals are registered, under the Veterinary and Para-Veterinary Professions Act 1982 (Act No.19 of 1982). Private veterinarians may be able to assist with euthanasia and assessment of sick birds, but may need to be cautious during infectious disease outbreaks, if they frequently handle domestic birds. Poultry industry and vets (to be kept informed).

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Chapter 4 – Risk Assessment

4.1. Disease threats/ hazards for South African seabirds

1. Parsons and Vanstreels (2016) performed a risk analysis (Southern African Seabird Colony Disease Risk Assessment) and compiled a list of disease hazards of high concern:

Table 4. The disease hazards of high concern for each seabird group.

Pathogen or disease	Penguins	Cormorants	Gannets	Terns	Gulls	Pelicans
Avian pox	○					
Herpesvirosis (S. American strain)	●					
Newcastle disease	●	●	●	●	●	●
Avian influenza (high pathogenicity)	●	●	●	●	●	●
Avian cholera	●	●	●	●	●	●
Chlamydiosis	●	●	●	●	●	●
Mycoplasmosis						○
Bacterial airsacculitis and pneumonia	○					
Bacterial gastroenteritis and colitis	○					
Aspergillosis	○	○	○	○	○	
Avian malaria	●	●	●	●		●
Babesiosis	○	○	○			
Coccidiosis		●				
Cryptosporidiosis	○					
Ticks	○					
Avian botulism	●	●	●	●	●	●
Marine biotoxins	●	●	●	●	●	●

Legend: ● – High outbreak impact score with medium/high probability of occurrence;
● – High outbreak impact score with low probability of occurrence;
○ – Medium outbreak impact with high probability of occurrence.

Based on the above table and recent HPAI outbreaks, disease priorities for this document comprise:

1. High pathogenicity avian influenza (HPAI, caused by a virus)
2. Avian cholera (*Pasteurella multocida* bacterium)
3. Newcastle disease (a virus)
4. Chlamydiosis (Psittacosis, caused by *Chlamydia psittaci*)
5. Avian malaria (a protozoan blood parasite, *Plasmodium spp*)
6. Coccidiosis (caused protozoan gut parasites)
7. Avian botulism (caused by botulinum neurotoxin produced by *Clostridium botulinum* bacteria)
8. Marine biotoxins (produced by marine algae)

4.2. Species of concern, based on the TOPMS Regulations (May 2017)

- Critically endangered
  - Damara Tern (*Sterna balaenarum*)
  - Leach's Storm-Petrel (*Oceanodroma leucorhoa)**
- Endangered
  - Bank Cormorant (*Phalacrocorax neglectus)*
  - Cape Cormorant (*Phalacrocorax capensis)*
  - Roseate Tern (*Sterna dougallii)*
  - African Penguin (*Spheniscus demersus)*
  - Cape Gannet (*Morus capensis;* still listed as Vulnerable under TOPS regulations but it is in the Endangered IUCN Red List Category)
  - Indian yellow-nose albatross (*Thalassarche carteri*)*

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- Vulnerable
  - Caspian Tern *(Sterna caspia)*
  - Great White Pelican (*Pelicanus onocrotalus)*
  - White-chinned petrel *(Procellaria aequinoctialis)**
  - Great winged petrel *(Pterodroma macroptera)**
  - Soft plumaged petrel *(Pterodroma mollis)**
  - Kergeulen petrel *(Lugensa brevirostris)**
  - Southern giant petrel *(Macronectes giganteus)**
  - Northern giant petrel *(Macronectes halli)**
  - Sub-antarctic (Brown) skua *(Catharacta Antarctica)**
  - Antarctic tern *(Sterna vittata)**
- Protected
  - Hartlaub's Gull *(Larus hartlaubii)*
  - Crowned Cormorant *(Phalacrocorax coronatus)*
  - Swift Tern *(Sterna (Thalasseus) bergii)*

* Species of concern at the sub-Antarctic Prince Edward Islands

4.3. Areas/ colonies of highest concern

Areas that should be prioritised for monitoring and risk mitigation are shown below in the table and map (). These are the BirdLife International Important Bird and Biodiversity Areas (IBAs) and areas that host TOPMS species. (References: "Important Bird Areas in Africa and Associated Islands: Priority Sites for Conservation (South Africa)" (Barnes et al., 2001) and species pages on the BirdLife Data Zone website, accessed in 2023.)

Table: Important Bird and Biodiversity Areas (IBA) in South Africa and the TOPMS species they host

Important Bird and Biodiversity Area	**TOPMS Species**					
	Damara Tern	**Bank Cormorant**	**Cape Cormorant**	**Roseate Tern**	**African Penguin**	**Cape Gannet**
Orange River Mouth	x		x			
Bird Island (Lambert's Bay)	x	x	x			x
Lower Berg river wetlands (Velddrif)	x	x	x		x	x
CNP and Saldanha Bay islands		x	x		x	
Dassen Island	x	x	x		x	x
Robben Island		x	x		x	
Simon's Town Penguin colony (including Boulders Beach)					x	
False Bay Nature Reserve			x		x	
Overstrand (Kleinmond, Bot River Estuary; Cape Whale Coast)		x			x	
Dyer Island		x	x		x	
Agulhas plain-Heuningnes Estuary	x	x	x		x	x
Maitland - Gamtoos coast			x			
Algoa Bay (Coast & islands)	x			x	x	x
Stony Point Nature Reserve*			x		x	

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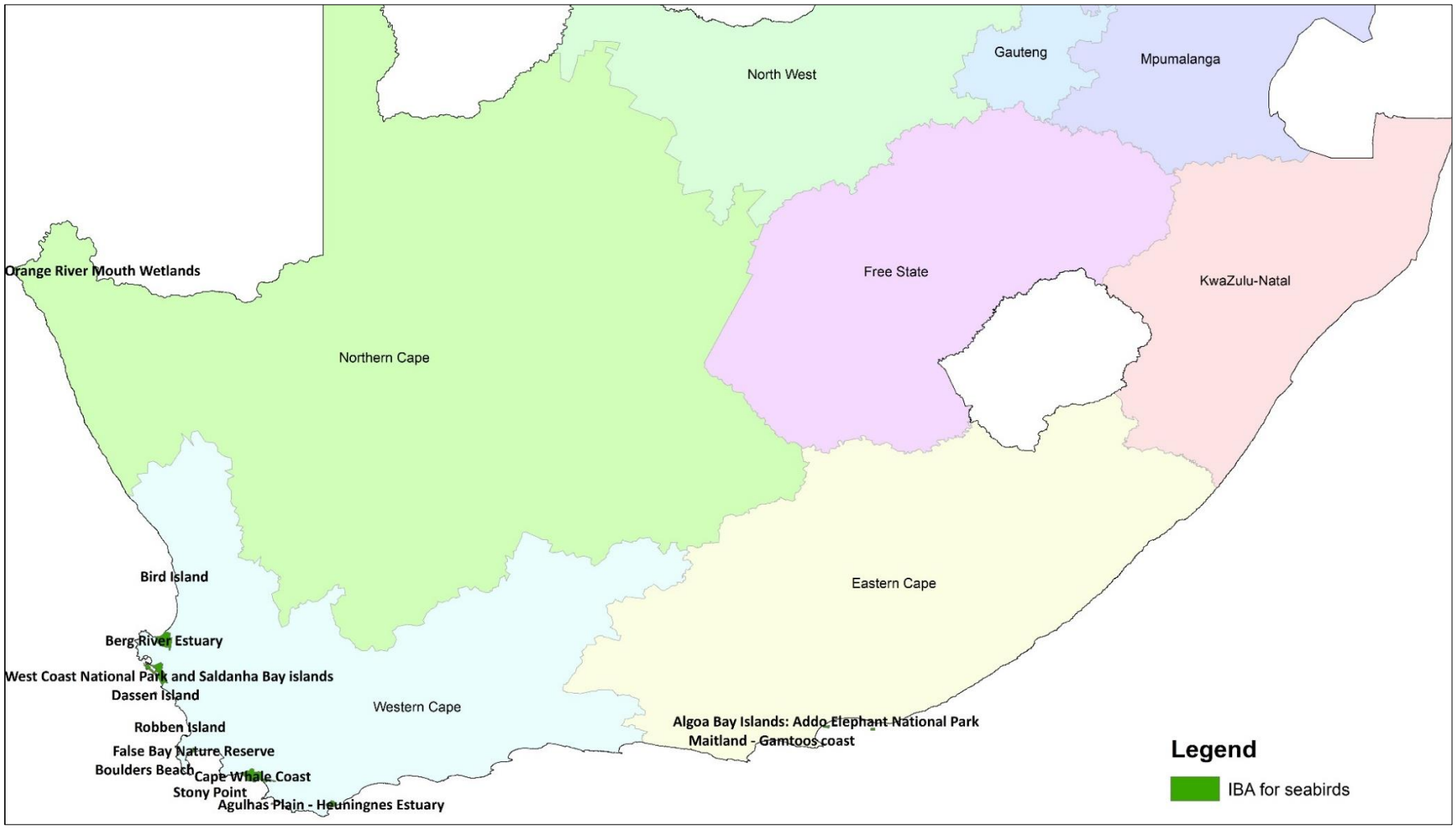


Figure 1(a): Important (Coastal) Bird and Biodiversity Areas of South Africa, according to BirdLife International (magnified Eastern Cape and southern Western Cape in (b)) (BirdLife International (2022) Important Bird and Biodiversity Area (IBA) digital boundaries: September 2022 version. BirdLife International, Cambridge, UK.)

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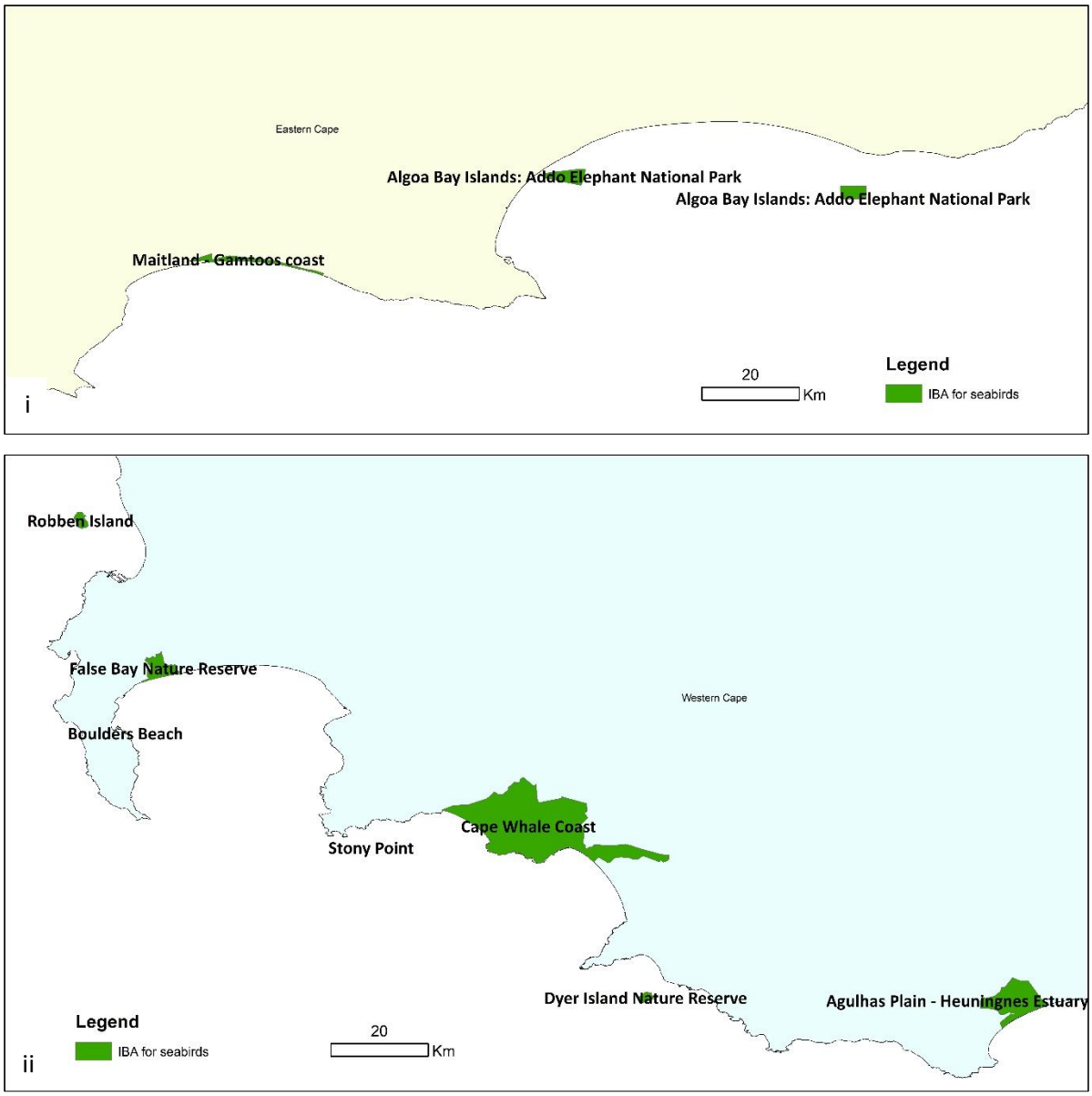


Figure 3(b): IBAs in Eastern Cape (i) and southern Western Cape (ii) (BirdLife International (2022) Important Bird and Biodiversity Area (IBA) digital boundaries: September 2022 version. BirdLife International, Cambridge, UK.)

A new African Penguin colony is being established in the **De Hoop Nature Reserve**. If this colony grows large enough it will also become an important breeding area for African Penguins and other seabirds.

Other factors to consider when prioritising colonies or aggregation sites for monitoring and risk mitigation:

- High colony density and colony size
- Mixing of species (biodiversity) especially birds migrating from Europe or Asia to Africa, birds flying between seabird colonies, landfill sites, estuaries and poultry farms
- Historical disease problems, e.g. previous disease incidents on Dyer Island
- Other risk factors e.g. V&A waterfront- additional potential human risk/ stressors,
- Remoteness, in that disease may not be detected or intervention may not be possible

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Chapter 5 – Disaster Risk Reduction

5.1. Direct disease prevention and mitigation measures

- Precautions should be taken to ensure that humans and domestic animals do not spread diseases into and between seabird colonies. A set of biosecurity guidelines are included in Annex 4.2, the HPAI management protocol, but specific protocols should be in place for each location.

- Research involving any samples taken from an animal requires permission from the national Director: Animal Health, DALRRD, under the Animal Diseases Act, Section 20. This process should assist with ensuring that the research will not contribute to disease spread. Application forms and further information are available online at https://www.old.dalrrd.gov.za/Branches/Agricultural-Production-Health-Food-Safety/Animal-Health/Epidemiology/researchapproval

- Research activities that require handling or sampling seabirds listed in TOPMS regulations need a research permit from DFFE. In addition, if the research takes place inside a national park or reserve, a research permit from the management authority is also required. This allows DFFE and management authorities to limit disturbance to seabirds, ensure the science aligns with the relevant legislation and enable direct communication between authorities and researchers, e.g. to advise on disease outbreaks and biosecurity measures.

- Colony managers and other staff working in seabird colonies should be adequately informed and trained on disease surveillance and disease mitigation activities.

5.2. General supportive conservation measures

General principles of habitat/ environmental conservation should be applied to maintain healthy populations and increase resilience to disease, e.g. preventing:

- Low food availability
- Climate change (affects disease vectors, food availability, breeding success and physiological stress on animals)
- Habitat loss
- Pollution - Refuse, Oil spills, Sewage (containing toxins, coliforms, Salmonella and pharmaceuticals)
- Predation
- Disturbance - by humans in breeding colonies and noise pollution and shipping at sea

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Annex 4.1

Chapter 6 – Preparedness planning

6.1. Early warning System (Surveillance):

6.1.1. Routine monitoring activities

- Baseline mortality and population census figures should be known or established so that abnormalities can be detected and investigated promptly.
- Wherever patrols take place, for whatever reason (e.g. law enforcement), personnel should be aware that reporting sick and dead birds is necessary and be trained to collect basic data.
- Each organisation should be able to report on:
  - monitoring activities and their frequency (active patrols/ reaction to reports/ incidental observations)
  - findings in terms of both lack of abnormalities and suspected cases.
  - It should be possible to distinguish a lack of incidents from a lack of effort.
  - Current coastal patrols are listed in Annex 4.1.2

- Minimum frequency of routine patrols
  - Every two weeks at identified high-risk sites, in low-risk seasons
  - Weekly in high-risk seasons (peak breeding) at high-risk sites
  - Monthly at low-risk sites

6.1.2. Data collection and management:

Data collected for sick or dead birds should at least include:

a) Date found/ observed
b) Exact location found (GPS coordinates or street address)
c) Species
d) Number of birds affected
e) Age category (chick/ juvenile/ adult)
f) If the bird was found alive or dead
g) If alive, what clinical signs was it showing?
h) If dead, in what state of decay was it found?
i) What was done with the bird?
j) Name and contact details of the observer in case further information is required
k) Photographs of dead birds should be taken from above and below, with the wings stretched out and tail spread, as well as a side view of the head. These are especially important if the species has not been identified with certainty.

Reporting

- Data should ideally be submitted via the web-based Seabird Avian Influenza Reporting Tool (ArcGIS Survey 123 Avian Influenza Reporting Tool designed and maintained by Branch: Oceans and Coasts, under the Oceans and Coasts Information Management System (OCIMS) Instructions for accessing the tool are included as Annex 4.1.3.
- Data can also be recorded in the template provided with Annex 4.1.3 and can be completed in Microsoft Excel or printed and completed on paper.

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- Another data collection system can be used, but the same data should be collected and available for easy collation with other organisations' data.

Data analysis

- The ArcGIS web map and dashboard can report/ display hotspots during disease incidents (See Annex 4.1.3)
- Data are freely available via the web map, with personal details excluded.
- Notification about abnormal events can be automated but continuous manual monitoring of the system is advised.

Personnel required

- Ideally, a person will be assigned to coordinate surveillance efforts, reporting, data analysis and feedback. The incoming data require checking and cleaning, and decision-makers must be notified of abnormal events.
- The surveillance coordinator is probably best employed via an NGO such as SANCCOB or BirdLife SA under short-term project funding for a PhD or Post Doctorate, so that the feasibility of a permanent post can be assessed.

6.2. Other early warning mechanisms

- Infectious diseases that have occurred in other parts of the country, continent or globe, which may move into seabird populations in the near future (e.g. new strains or high incidence of avian influenza in other countries).
- Occurrence of identified risk factors such as acute sea level temperature increases (marine biotoxins), and high rainfall leading to increased mosquito breeding (associated with malaria).

6.3. Guidelines for preparedness activities

- Compilation of site-specific contingency plans and SOPs
- Equipment and other resources, should be kept on standby
- Guidelines for accessing emergency funding should be available
- Annual training/ refresher programme to maintain awareness
- Annual exercises to practice response
- Annual updating of the contingency plans

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Annex 4.1

Chapter 7 – Response

7.1. Outbreak investigation

1. Establish a case definition/ condition of interest (exactly what is it that you are concerned about and that you want people to look for?)
2. Confirmation/ verification of outbreak/ event (What is normal? Is there really a problem? Has detection effort changed and affected what is being reported?)
3. Describe the outbreak in terms of numbers (proportion affected), time (when, how long, other events), space (describe the locations, environment, other species) and population affected (species, age/ life stage, sex)
4. List possible causes/ theories of cause/ route of exposure
5. Determine risk factors associated with cases (relate to space/ time/ population: who, what, where, when)
6. Perform clinical examinations, post-mortem examinations/ necropsies, take samples, do testing
7. Tentative diagnosis
8. Management/ response plan
9. Test hypotheses
10. Refine management
11. Communicate with the public

These steps can be done simultaneously or in another order. Some steps may proceed more rapidly if a diagnosis is more obvious or easily identified (Arthur L. Reingold, 1998; Preece et al., 2017)

Determining the cause of a mass mortality or disease incident detected during surveillance:

- All available information should be gathered and organised to ensure the cause is accurately identified.
    - Reports from the public should be confirmed wherever possible, and precise and detailed records should be kept.
    - Speculation and assumptions about the cause should be avoided until all information has been assembled.
- The same information as for single mortalities should be collected but extra information is needed to help with identifying the cause e.g. environmental conditions or recent weather events, human activities. **Refer to the checklist in Annex 4.1.4**
- If no obvious fatal injury is visible, then collect the bird (s) and send the whole carcass or samples for analysis.
    - Try to find fresh carcasses, as identifying the cause of death becomes more difficult as carcasses rot. A fresh carcass may still be stiff with rigor mortis, the eyes may be partially sunken but not completely sunken or dried out. Carcasses should be sent for examination within three days of death but preferably within eight hours.
    - Carcasses for post-mortem examination should not be frozen but should be kept cool by placing them in a fridge. Staff should be supplied with a refrigerator or a freezer in which to store carcasses.
    - Staff should receive basic training and equipment, including protective clothing, for opening and photographing carcasses and for taking samples that can be stored more easily.

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- If possible, engage a professional pathologist to assess carcasses and samples, or submit carcasses to a local vet or institution capable of performing necropsies.
- Once the cause has been determined, sampling may become unnecessary, but it may still be necessary to confirm that the disease is affecting new sites and species. There may also be funding available for a higher number of samples to assist with gaining a better understanding of the disease.

7.2. Coordination and stakeholder communication channels

- Assign a coordinator for centralised reporting and decision-making and assign responsibilities clearly.
- Outbreak response coordination for major or widespread incidents can be implemented through a joint operations centre (JOC) based at a Provincial or District disaster centre or through a smaller group, in proportion to the response needed.
- Prepare a list of stakeholders that need to take action and/or be informed, and update contact details at least once a year- refer to Chapter 3: Institutional Capacity for guidance and Annex 4.1.1 for contact details. Involve all who are responsible for conservation areas, beaches etc. and for different functions- disease control, waste management, field staff etc.
- Employ instant messaging (e.g. WhatsApp) groups and/or email distribution lists and regular meetings to keep everyone informed and discuss challenges AND solutions.
- Ensure that at least one person from each organisation is included in meetings and updates and that they will communicate within their organisation when necessary.

7.3. Media/ public communication

- Identify communication staff from each organisation involved and a coordinator
- Ideally issue single, joint media statements from a single point (see Annex 4.1.5 for a template)
- The aim is a cohesive, consistent narrative to the public that informs them of what the problem is, reassures them that it is being dealt with, makes them aware of any danger and instructs them on what their actions should be, if applicable.
- Ensure that information is adequately and timeously distributed and pre-empts or corrects dominating false information.
- Ensure that media statements are clearly written and avoid jargon, abbreviations and technical terms
- It is important to do regular updates if the incident is prolonged and then a closing media release to indicate resolution.
- Prepare Frequently Asked Questions (FAQ) for different diseases/ situations (see Annex 4.1.6)
- Establish the website and/or social media capacity for each organisation and employ these to improve communication (consider links to other organisations' websites).

7.4. Outbreak/ incident management

It is important to first assess the potential for actions to contribute to disease control objectives and to balance any advantages against the resources required and the potentially harmful effects of

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response actions on seabirds and their environment. Guidelines specific to each disease will be included in Annex 4.2.

7.4.1.Carcass removal and disposal

Collection of carcasses may not be necessary or justifiable for all situations or locations. Collecting carcasses or sick birds may cause more harm than good if a breeding colony is disturbed or habitat damaged, so this must be considered for each location. Additionally, handling and transporting carcasses may contribute to the spread of disease and this risk must be adequately managed. However, carcasses may also cause other problems such as aesthetic effects on public beaches, public health risks or providing a food source for pests and/ or scavengers.

Carcass removal should only be done if all the following criteria can be met:

- A legal, safe and effective carcass disposal option is available.
- Required equipment is available, including storage containers/ bags, vehicles, tools and possibly earth-moving equipment.
- Personnel are available or can be sourced and trained to collect carcasses.
- Carcass removal can be done with minimal risk to people. Risks can be related to dangerous terrain or a pathogen with a high potential to transmit to humans. Anyone handling potentially infected carcasses should wear suitable protective clothing. See Annex 4.1.7 for PPE recommendations.
- Carcass removal and disposal can be done without increased risk of disease spread to new areas or species.
- Fresh carcasses can be collected or frequent collection can be organised.

Legal requirements for carcass handling and disposal:

- Environmental legislation: Official approval will be required from a provincial or municipal department because infectious carcasses or those containing a toxin are classified as hazardous waste. They may not be disposed of at a landfill site. The provincial Directorate: Waste Management (see contact list and Annex 4.1.8 for forms) may be applied to for limited, emergency approval, under section 30A of the National Environmental Management Act (NEMA) (Act No. 107 of 1998; Control of emergency incidents).
- Plans to incinerate carcasses must be discussed and confirmed with a Municipality's Air Quality Officer (AQO), though the provincial Director: Air Quality Management may be able to assist. The Atmospheric Emission Licenses of licensed facilities are regulated by different Municipal AQOs, and not by Province.
- Provincial Veterinary Services must approve transport and disposal where a Controlled Disease (Animal Diseases Act) is involved.

Carcass disposal methods

The best carcass disposal option for each location should be identified in advance and plans made for different volumes of carcasses and ideally, for contagious (where transport of carcasses may spread disease) and non-contagious diseases.

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Carcass disposal options include:

A. Burial: sufficient space and suitable substrate is required to ensure that the carcasses will not cause contamination of soil or water sources. Ensure scavengers (human and animal) cannot uncover carcasses.
B. Composting (a carcass treatment method)- sufficient plant material is required to achieve the correct carbon-nitrogen ratio and the correct moisture level. Expert involvement is recommended. Pathogens can be inactivated and a useful by-product created.
C. Incineration/ burning may be a better option for islands, especially if outbreak is isolated to the island. Requires approval from Air Quality Control on mainland South Africa and special permission from Prince Edward Island management.
    a. Incineration (in an incinerator) on-site or outsourced. The processing capacity of incinerators can be limited.
    b. Burning- sufficient fuel and airflow (possibly burning on a platform) are required to maintain sufficient heat. Ensure feathers are contained and are not distributed by updrafts. Do not try to burn too many carcasses at once.
D. Rendering (a form of cooking) is a theoretical possibility in South Africa but there are not many facilities.

Note: Staff collecting carcasses must be able to identify species, age etc. and have the tools to easily keep records of numbers. Basic records can be kept of date, location, species and numbers but any changes in trends should be flagged and reported.

7.4.2.Managing sick birds

Sick birds may be a source of infection or toxin for other birds and will be suffering. However, it may not always be feasible to collect and care for them or to euthanise them.

Options for dealing with sick birds include:

Treatment

- If a specific treatment exists or if supportive care will help them recover
- If a facility and personnel (rehabilitation centre) are available and willing to carry out treatment
- If funding is available for rehabilitation
- If sick birds can be collected without disturbing a colony or spreading disease
- If transporting and treating the birds will not pose an unacceptable risk for disease spread (good biosecurity measures can mitigate this risk)
- If the affected species tolerates transport and rehabilitation

Euthanasia (see Annex 4.1.9)

- If treatment is not possible or effective
- To prevent suffering
- To slow disease spread
- If sick birds can be collected without disturbing a colony or spreading disease
- If capturing, transporting and euthanising the birds will not pose an unacceptable risk for disease spread (good biosecurity measures can mitigate this risk)
- If sufficient competent personnel and funding are available

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Annex 4.1

No action

- If treatment and euthanasia are not possible, effective, or safe.

7.4.3.Biosecurity measures (as disease prevention and control)

Biosecurity consists of managing animals, people and objects (fomites) that could contribute to the spread of a pathogen or toxin. Annex 4.2 has an example of a protocol.

Preventing people from being a disease source

- Staff- minimise the number of staff that access contaminated areas and have contact with sick birds. Create separation between staff from worse and less affected areas. Supply disinfectant and sufficient protective clothing.
- Researchers- limiting access to affected sites should depend on the importance of the research (consult DFFE Oceans and Coasts/ Seabird Technical Team) and severity of the outbreak, as well as which other colonies they have visited/ will visit in terms of other colonies' disease status and risk. All research permits should be issued only on provision of a detailed biosecurity plan, by the principal investigator.
- All people accessing affected colonies
    - Ensure separate clothing or protective clothing and footwear and equipment for different areas/ colonies
    - Soiled clothing & footwear should be removed before leaving a contaminated area (and before entering a vehicle)
    - Contaminated clothing must be handled so that it does not cause further contamination- buckets or bags for transport/ disposal/ disinfection of contaminated protective clothing should be available.
    - Apply disinfectants to hands and arms that could be contaminated and disinfect [clean] clothing.
    - Disinfection can be used as long as it can be done effectively, as described below.
    - Do not enter a property containing poultry for three days afterwards and shower and change clothes before handling any bird or making contact with any person who works with birds.
- Visitors- assess contamination of areas to which visitors have access and available mitigation measures, the zoonotic potential of the pathogen involved, the potential for visitors to spread pathogens to other wild or domestic birds, the effect of visitors causing additional disturbance to birds, visible signs of disease, the effect on visitors/ and the reputation of the protected area or facility.

Disinfection of potential fomites (equipment, vehicles and other contaminated objects)

- Disinfection is a measure to mitigate risk. The correct disinfectant must be used for the pathogen in question, it must have been stored correctly and must not be expired, it must be applied at the correct concentration and for the right contact time and only on clean objects.
- Footbaths are a possible means of disinfecting footwear. However, maintaining the correct concentration and cleanliness is difficult and sufficient contact time may be impossible. Additionally, some disinfectants are inactivated by sunlight. Mechanical cleaning and

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removal of dirt may be more effective, and changing footwear may be necessary. Removed footwear can then be soaked in disinfectant for sufficient time.

- More detail is supplied in Annex 4.2.

Birds and animals

- Use disposable or cleanable boxes for transporting sick (or dead) birds and ensure that all vehicles they enter and anywhere they are kept are cleaned and disinfected afterwards. Members of the public should be advised not to touch sick or dead birds, but if it is necessary, they should at least use newspaper or plastic bags as makeshift gloves to handle carcasses and keep the bird well away from the face.

7.4.4. Vaccination

Vaccination is only an option if:

- Effective vaccine(s) exists for the pathogen
- Effective and safe vaccine(s) exists for the species at risk
- Vaccine is commercially available in South Africa or can be imported/ manufactured with special permission (SAHPRA and DALRRD- Directorate: Agriculture Inputs Control (Act 36) and Directorate: Animal Health)
- Vaccine is approved/ can be approved for the species and situation in question
- Enough doses of vaccine can feasibly be administered to enough birds without causing excessive disturbance or damage

7.5. Determining the end of an outbreak

A continuing downward trend in cases over a period defined for each disease should indicate that an incident is coming to an end, and efforts can start to ease. Once no cases have occurred for that period, intensive monitoring can be suspended but regular monitoring should continue.

A media statement should be published to inform the public and role-players that the incident is considered over.

Chapter 8 – Recovery (Demobilisation)

When response to a disease or mass mortality incident is no longer required, personnel and equipment can be returned to normal functions. However, cleaning and disinfection of all equipment and clothing should be done, repairs made where necessary and preparation made for the next incident:

- Consumables should be restocked
- A debrief session should be held within three months of the end of the incident to analyse the response and update the contingency plan where necessary
- Routine monitoring should continue and censuses should be conducted carefully in order to estimate the long-term effect of the incident on the population/ ecosystem.

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List of Annexes

4.1.1. Contact list (pg 225)

4.1.2. List of organisations performing monitoring and patrols (pg 228)

4.1.3. Case reporting template/ DFFE app instructions (pg 229)

4.1.4. Mortality investigation form (pg 261)

4.1.5. Template media statement (pg 263)

4.1.6. Example: disease information/ Frequently Asked Questions (FAQ) sheet(s) for the public (Western Cape Department of Agriculture) (pg 265)

4.1.7. Specifications for PPE (pg 267)

4.1.8. Waste Management Annexes A & B (pg 269)

4.1.9. Euthanasia of seabirds (SANCCOB) (pg 273)

4.2. Disease protocols

High pathogenicity avian influenza (pg 278)

4.3. Coastal seabird contingency plans for individual protected areas

Protocol for the management of high pathogenicity avian influenza (HPAI) for Marion Island (pg 294)

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Contact List

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South African seabird disease outbreak preparedness plan: May 2024

Annex 4.1.2: Current coastal patrols/ surveillance activities and organisations involved (June 2022)

1. SANParks- colonies monitored daily, islands on West Coast not manned year round - rarely visited outside the breeding season, but visited by DFFE & academic researchers, and Pelican Watch
2. Coastal and marine monitors (DFFE) – regular patrols but long stretches of beach- whole SA coastline (34 monitors in total)

Western Cape

3. CapeNature- colonies monitored daily. Roosts visited less regularly but there is capacity to arrange more regular patrols for hotspots. Stranding networks and estuary forums do cover some of the estuaries
4. Robben Island- SANCCOB ranger does daily monitoring
5. City of Cape Town- Simon's Town patrolled daily but the whole coast is monitored
6. City of Cape Town Waste Management- do some beach cleaning and have a contractor for carcass removal
7. Shark Spotters- False Bay (Fish Hoek, Muizenberg, St. James, Caves and Clovelly and Monwabisi (seasonally) and Plettenberg Bay https://sharkspotters.org.za/
8. Two Oceans Aquarium Marine Wildlife Management Programme - daily patrols at Waterfront and harbour
9. Blouberg conservation authority 021 441 8193
10. Berg Estuary- 2-3 times a week depending on capacity
11. Overstrand Local Municipality- do patrols and help with Klein and Bot estuaries
12. Overberg District Municipality- one official does monthly patrols, 330 km covered (some driven. Reports to Overberg Stranding Network
13. Plettenberg Bay Stranding network
14. Mossel Bay (SMART) - active stranding network, publish a quarterly report
15. Nature's Valley Trust
16. WWF marine & Coastal Community Monitors- Kogelberg https://www.wwf.org.za/our_news/our_blog/the_monitors_daily_walk_for_the_oceans/

Eastern Cape

17. Bayworld- monthly patrols: two sections east and west of Gqeberha, in collaboration with NMU. Data goes to NMU and to SANParks for Addo section. To the west, cover 20km coast in St Francis Bay (the bay itself), and to the east, 40 km in Algoa Bay.

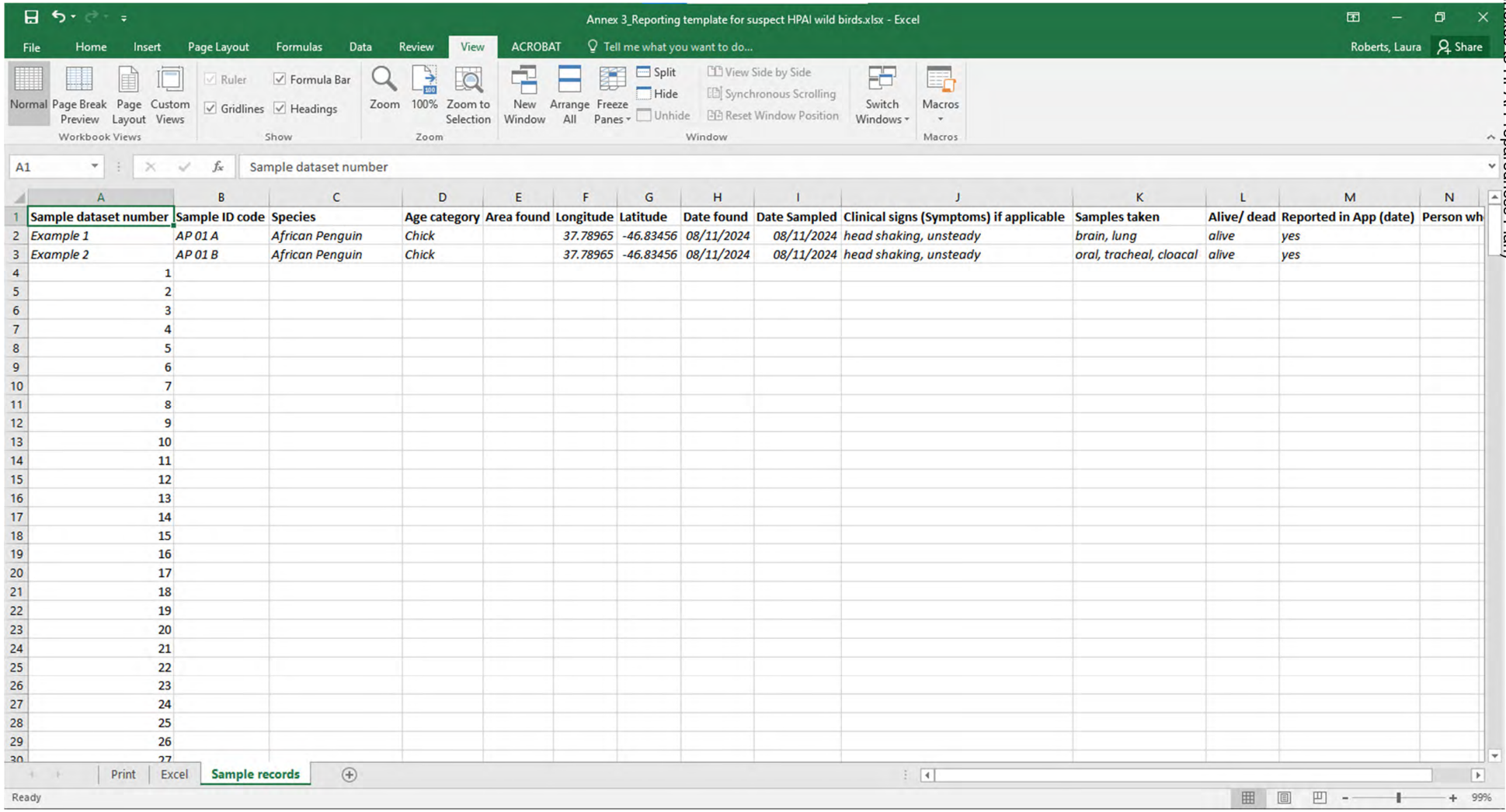
KwaZulu-Natal

18. KZN Ezemvelo Wildlife, Wild Oceans http://www.kznwildlife.com/Reseach%20Contact.html https://wildtrust.co.za/

Northern Cape- some coastal monitors- province does biannual monitoring

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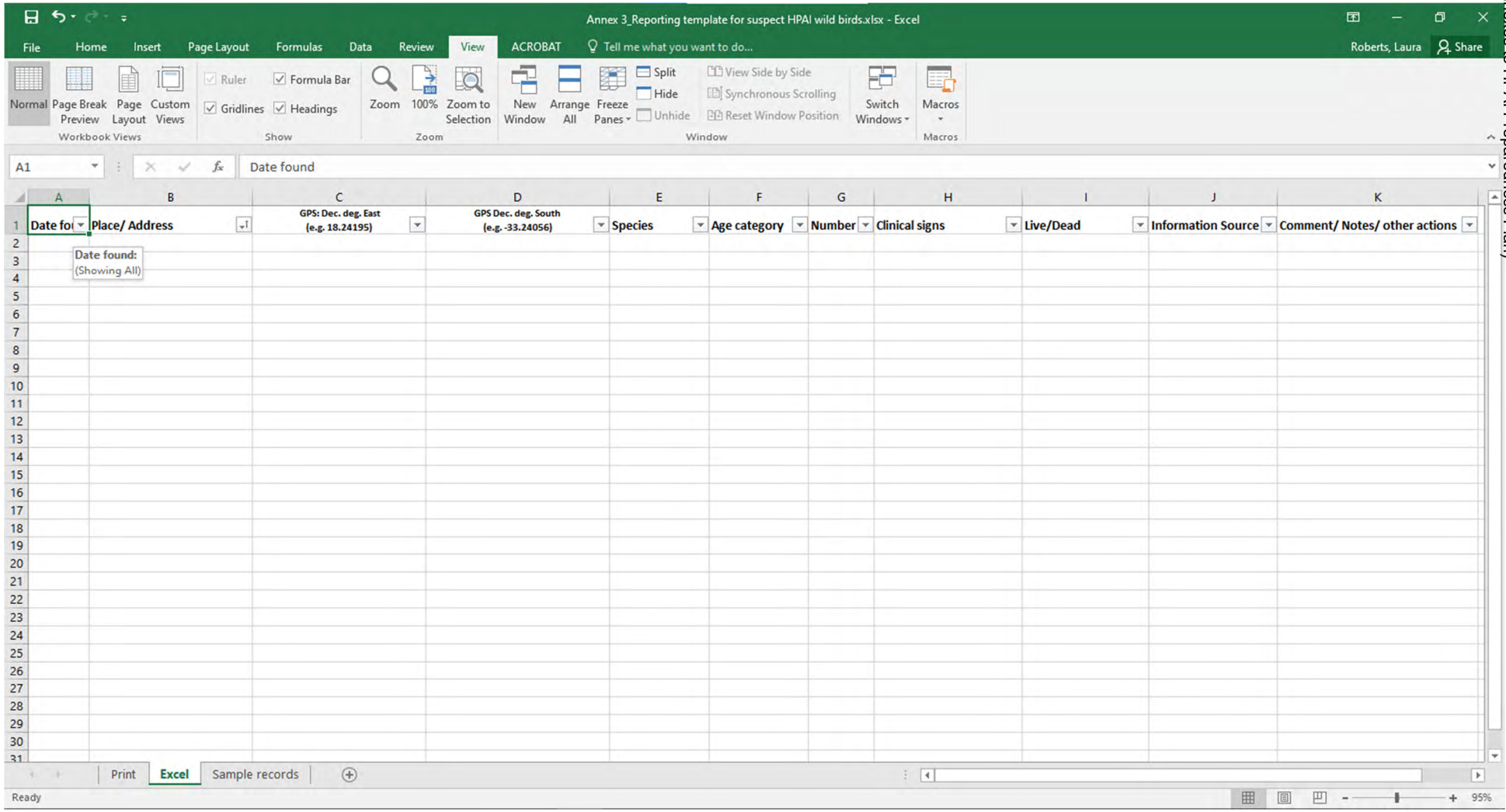
Annex 4.1.3: Sample records



Sample dataset number	Sample ID code	Species	Age category	Area found	Longitude	Latitude	Date found	Date Sampled	Clinical signs (Symptoms) if applicable	Samples taken	Alive/ dead	Reported in App (date)	Person wh
Example 1	*AP 01 A*	*African Penguin*	*Chick*		*37.78965*	*-46.83456*	*08/11/2024*	*08/11/2024*	*head shaking, unsteady*	*brain, lung*	*alive*	*yes*	
Example 2	*AP 01 B*	*African Penguin*	*Chick*		*37.78965*	*-46.83456*	*08/11/2024*	*08/11/2024*	*head shaking, unsteady*	*oral, tracheal, cloacal*	*alive*	*yes*	
1													
2													
3													
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Annex 4.1.3: Surveillance/ outbreak data collection template



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Surveillance/ outbreak data
(v. 2024.04)

Date (DD/MM/YYYY)	Dec. deg. East (e.g. 18.24195) OR area name/ beach code/ waypoint number	Dec. deg. South (e.g. -33.24056)	Species	Number	Clinical signs	Name & contact details (Email/ Cell) of person reporting	Comments: lab tests / other healthy bird species seen / other relevant information

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When completed please return to your supervisor OR scan/phograph and email to laura.roberts@westerncape.gov.za

How to: The Avian Influenza Reporting Tool for coastal birds



forestry, fisheries & the environment

Department:
Forestry, Fisheries and the Environment
REPUBLIC OF SOUTH AFRICA



Western Cape Government

FOR YOU

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- For online form and access via the app

https://arcg.is/1585011

- For the Webmap/ data access

https://dea-surveys.maps.arcgis.com/apps/webappviewer/index.html?id=af4cb7f7ed4e4442ae861cfb60e128e6

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Use one of these web browsers

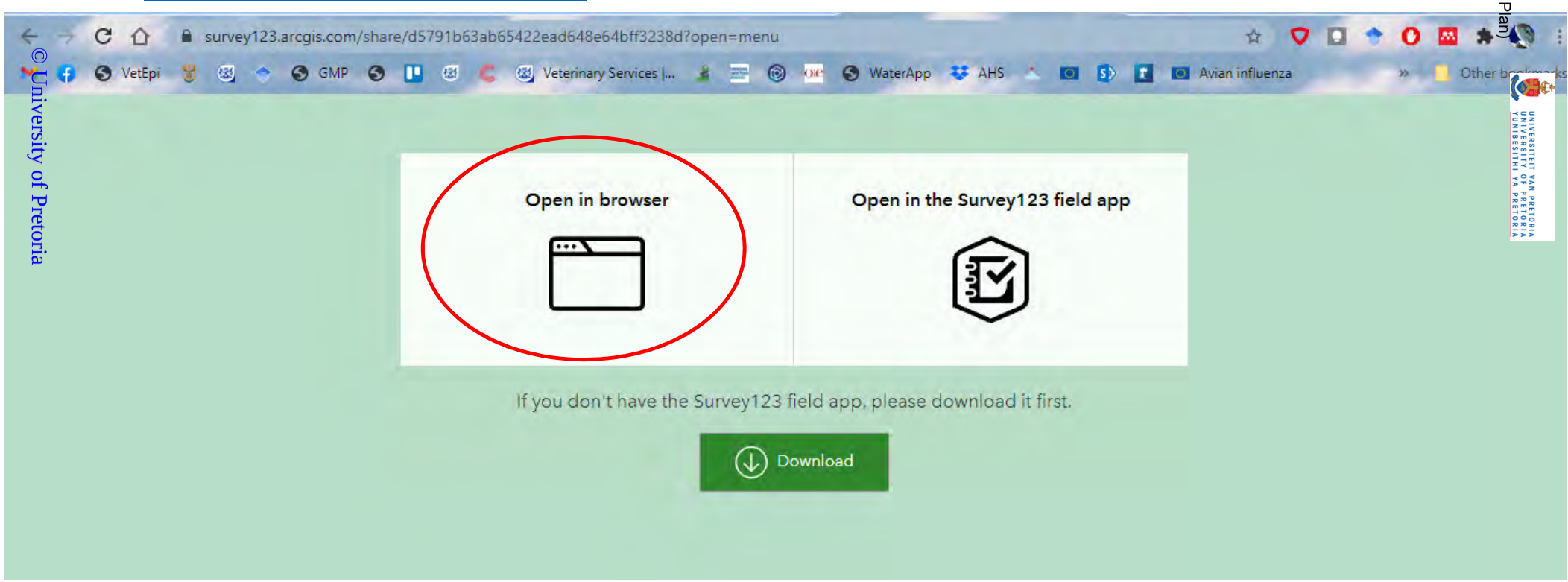
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- Firefox
- Apple Safari
- Microsoft Edge



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Open the form

- https://arcg.is/1585011



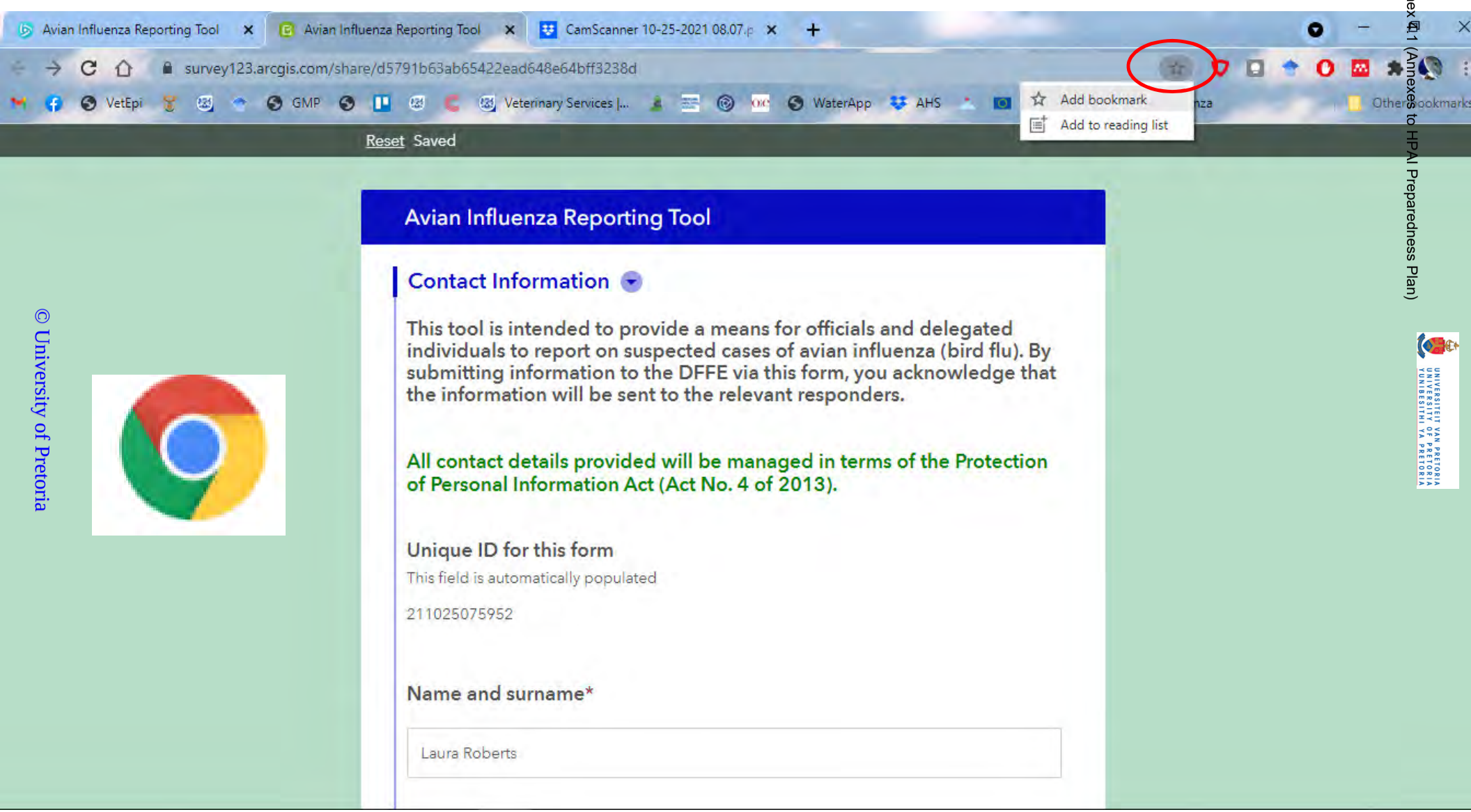
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To access the online form (cont.)

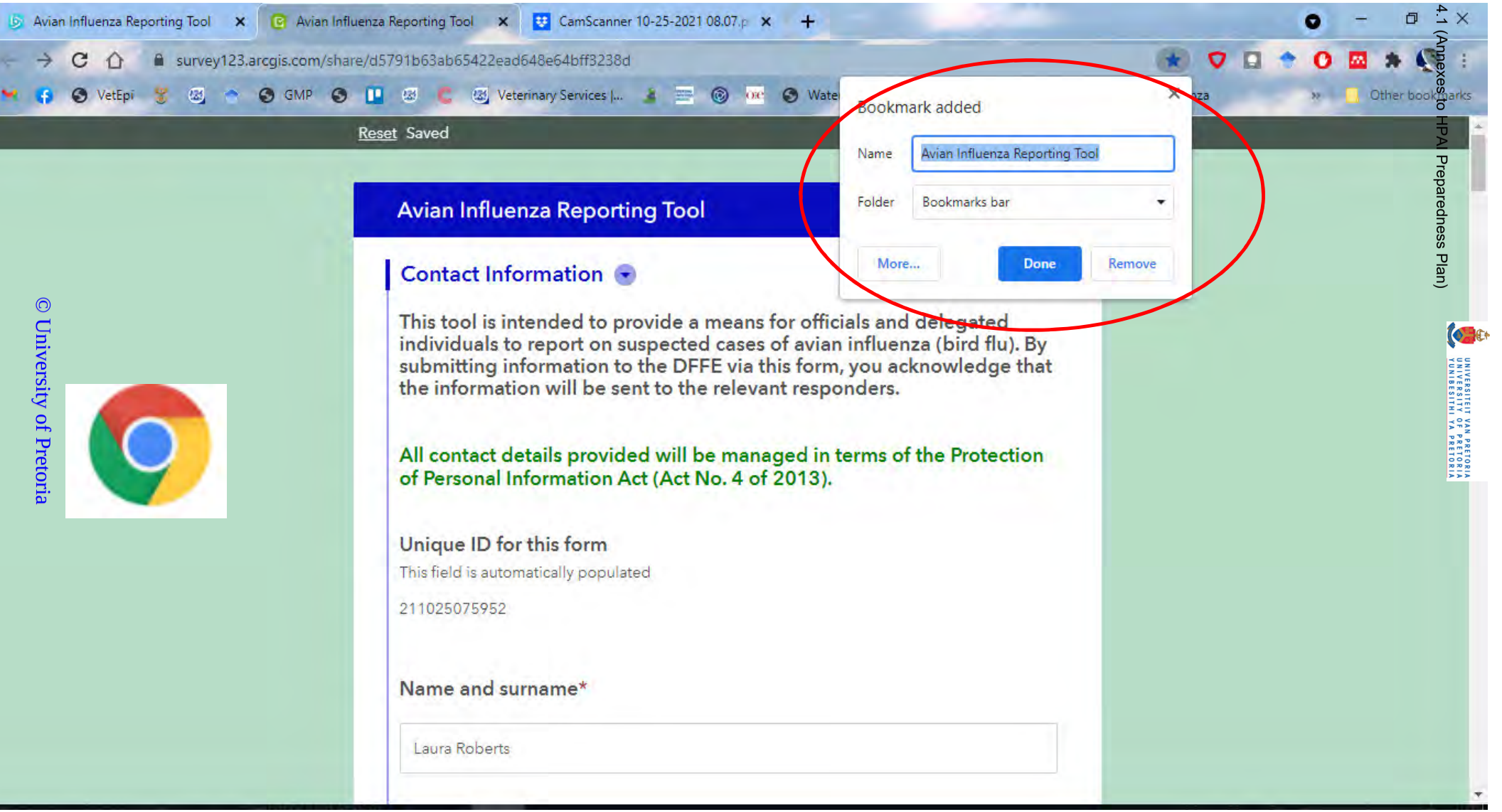
- Save a bookmark for easy access in future…



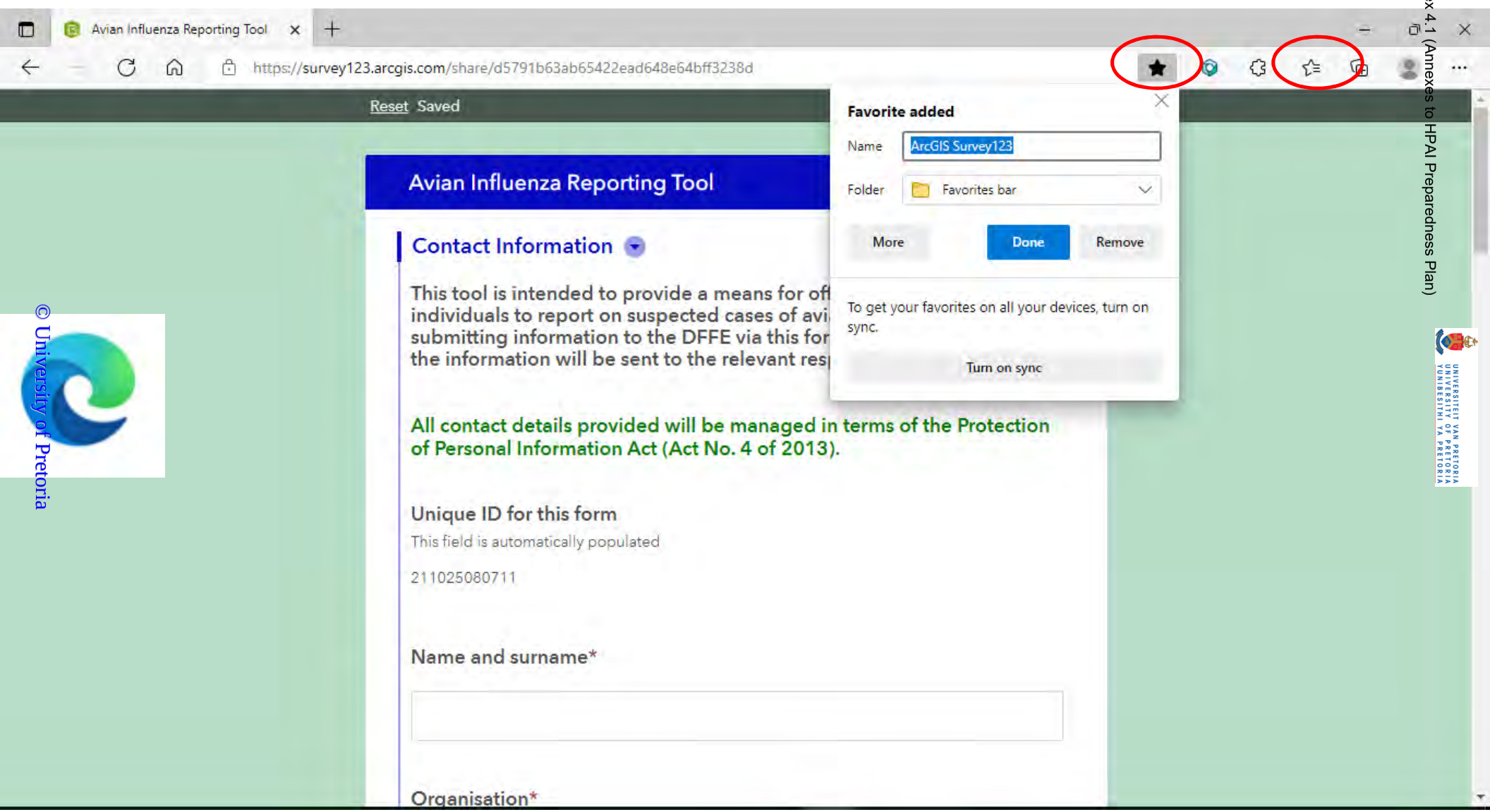
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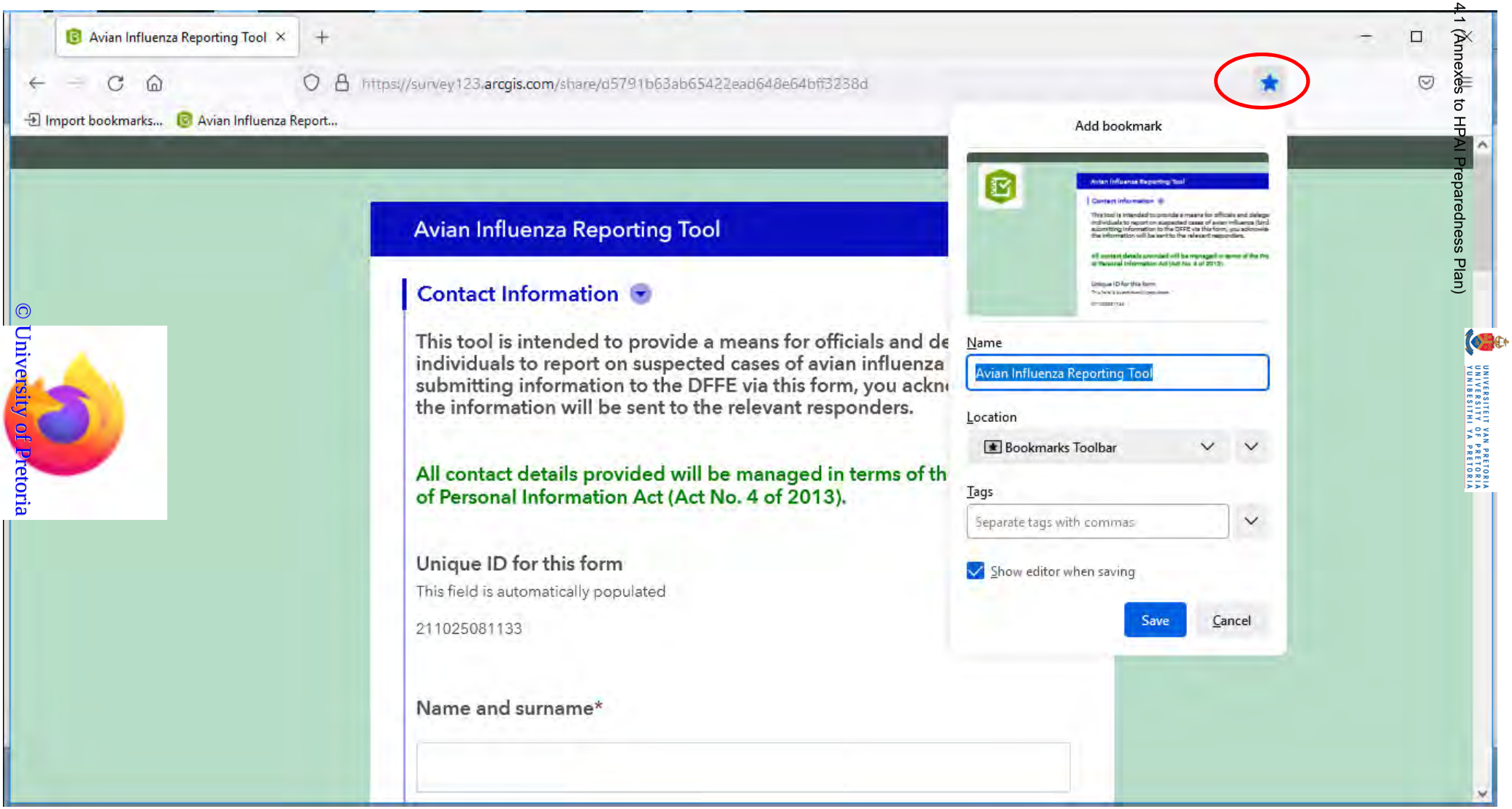
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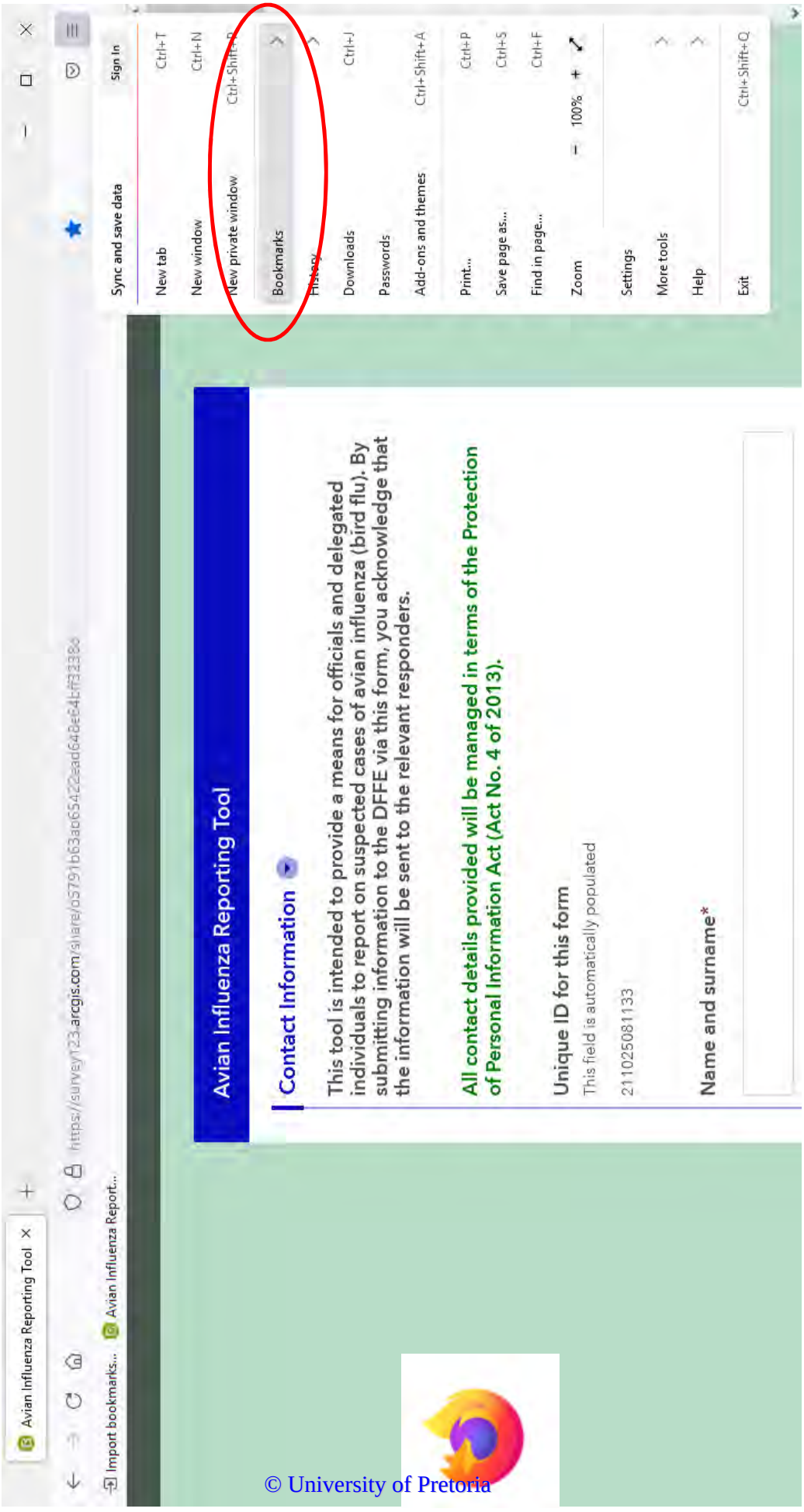
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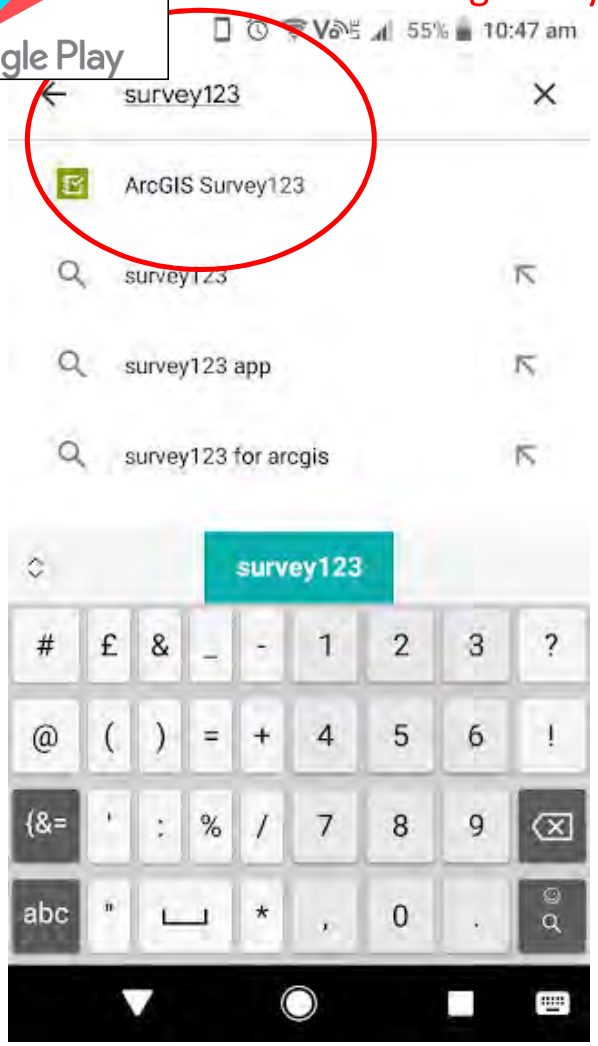
Do download the app on your cell phone

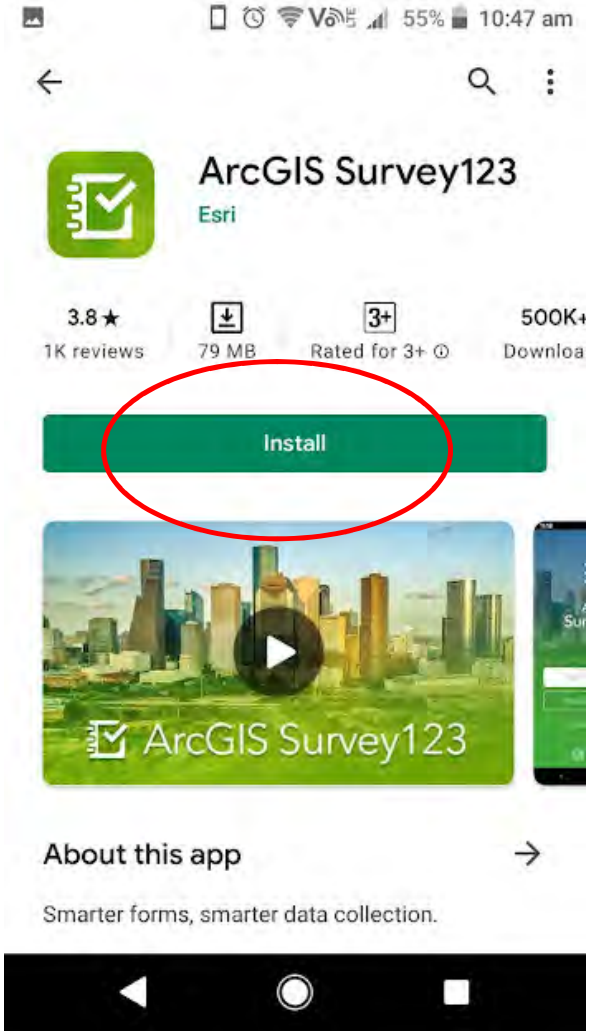
- Open Google Play
- OR Open Apple App Store

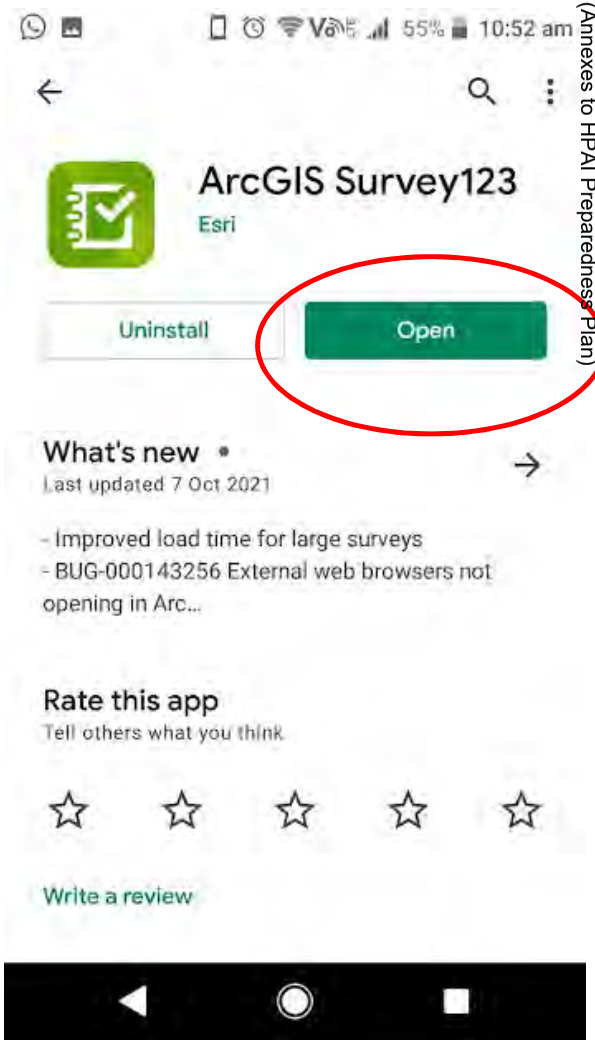
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Google Play

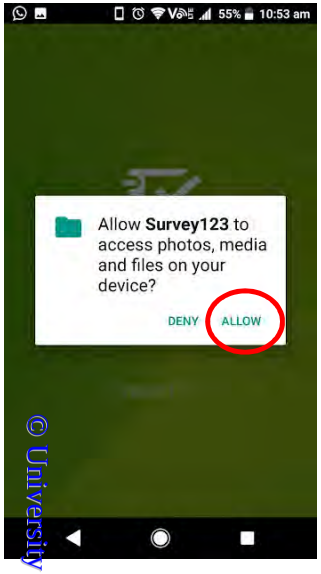
Search in Google Play

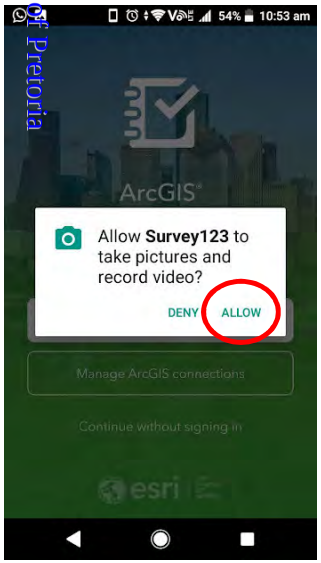


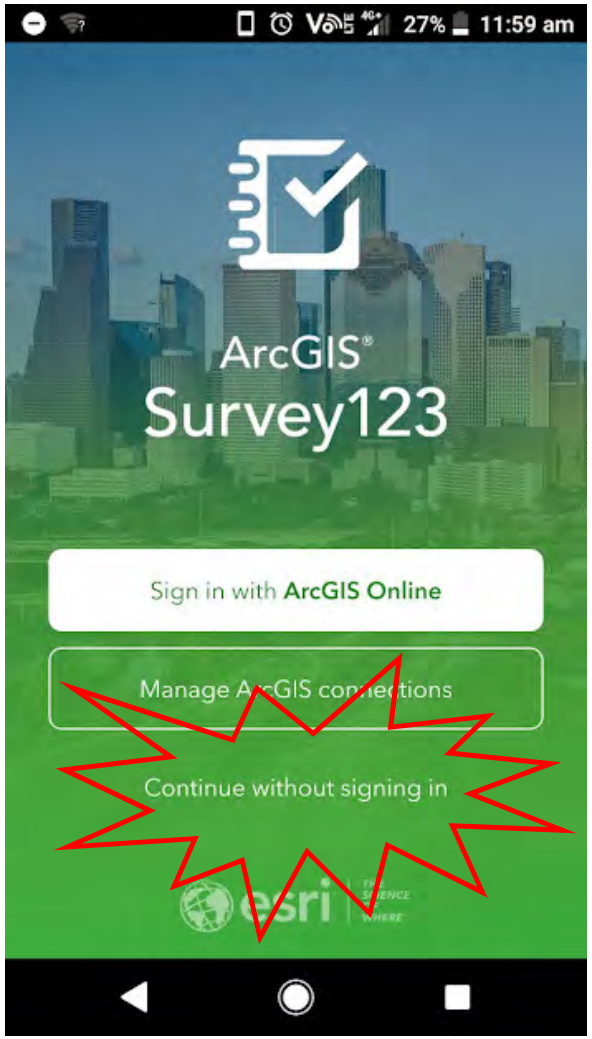


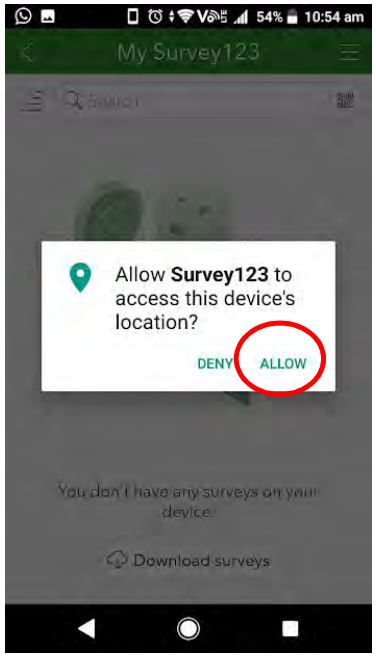


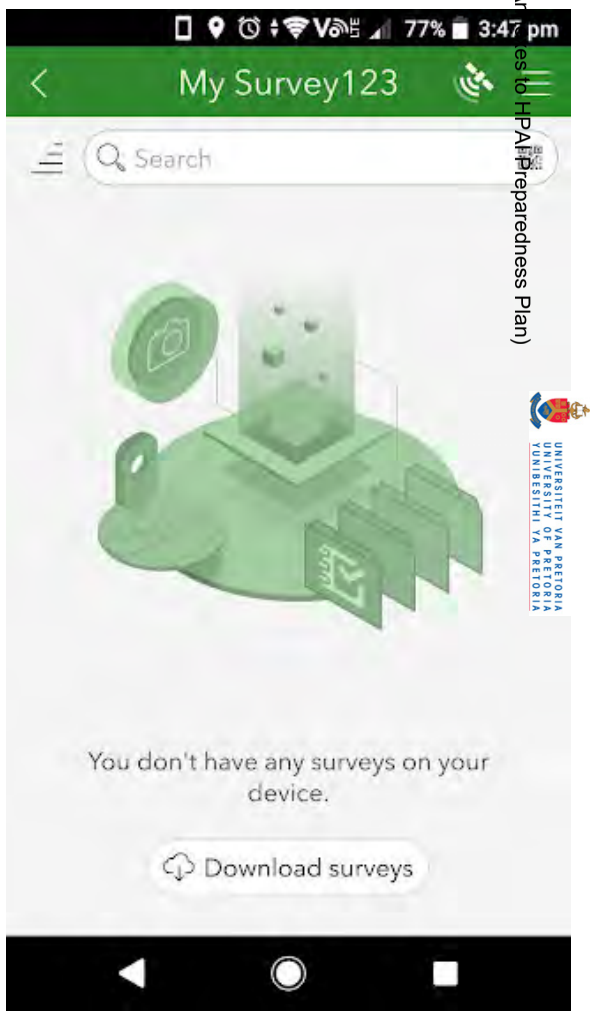
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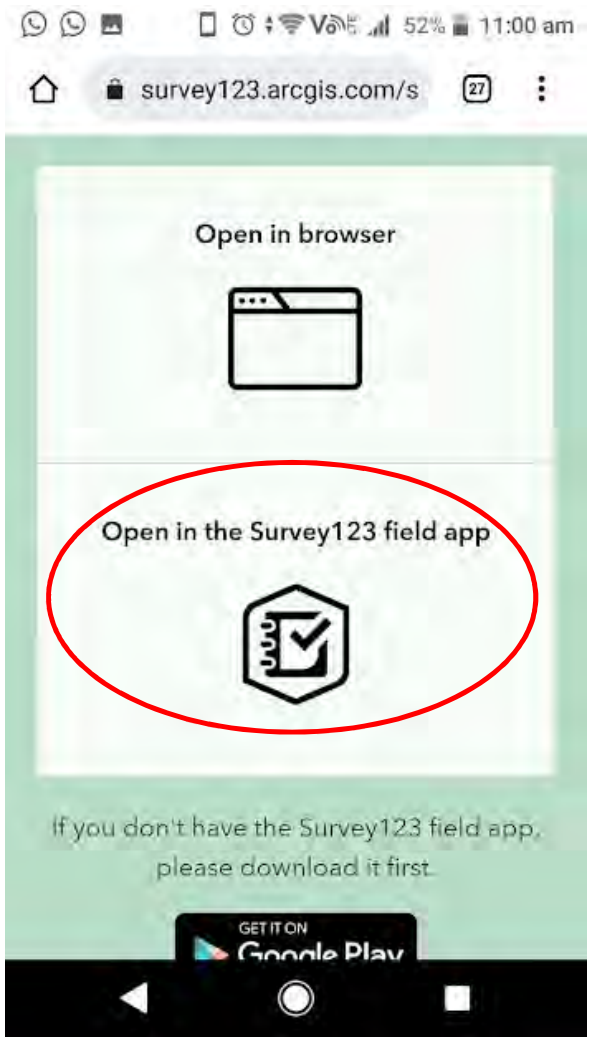




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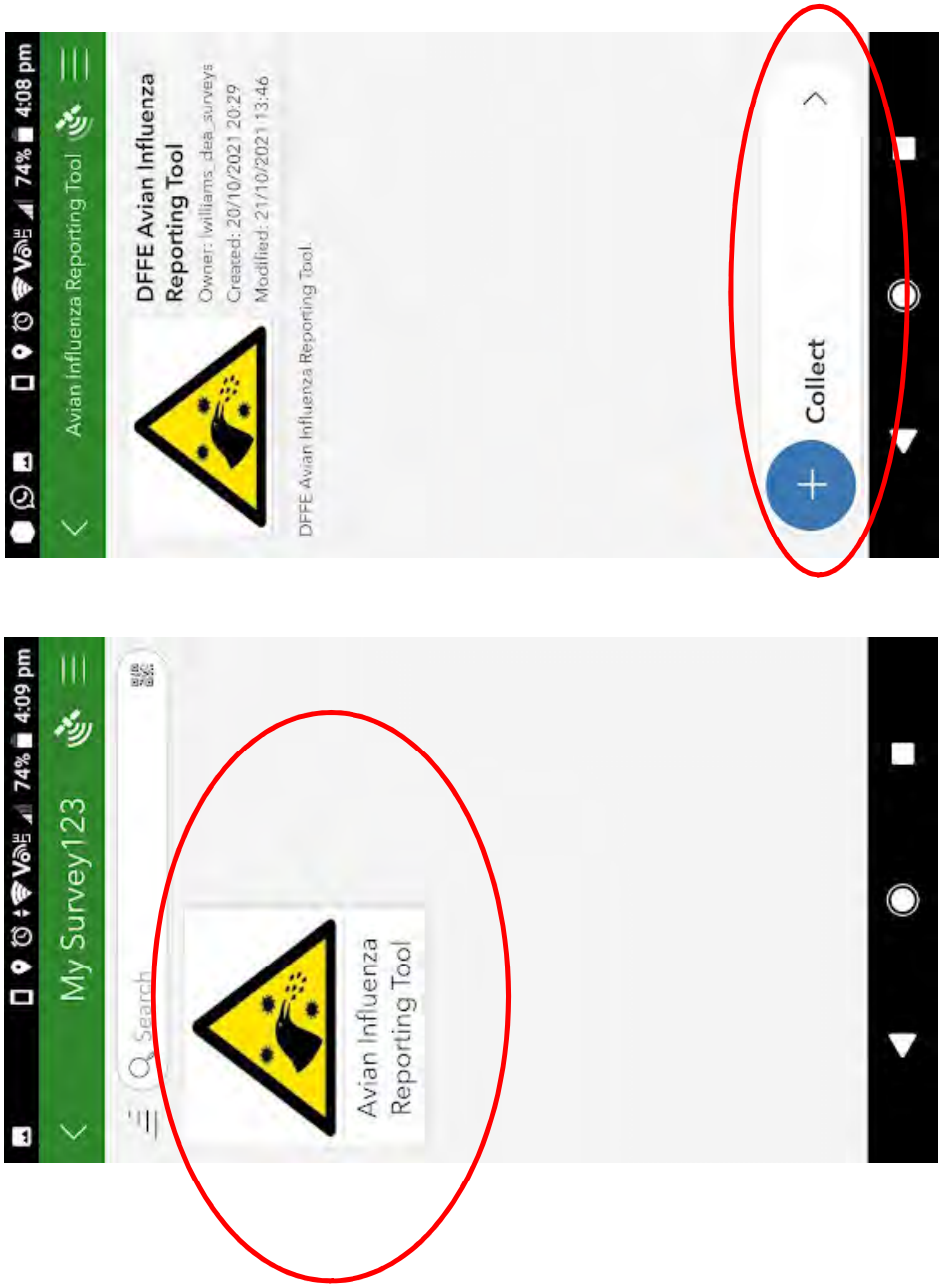
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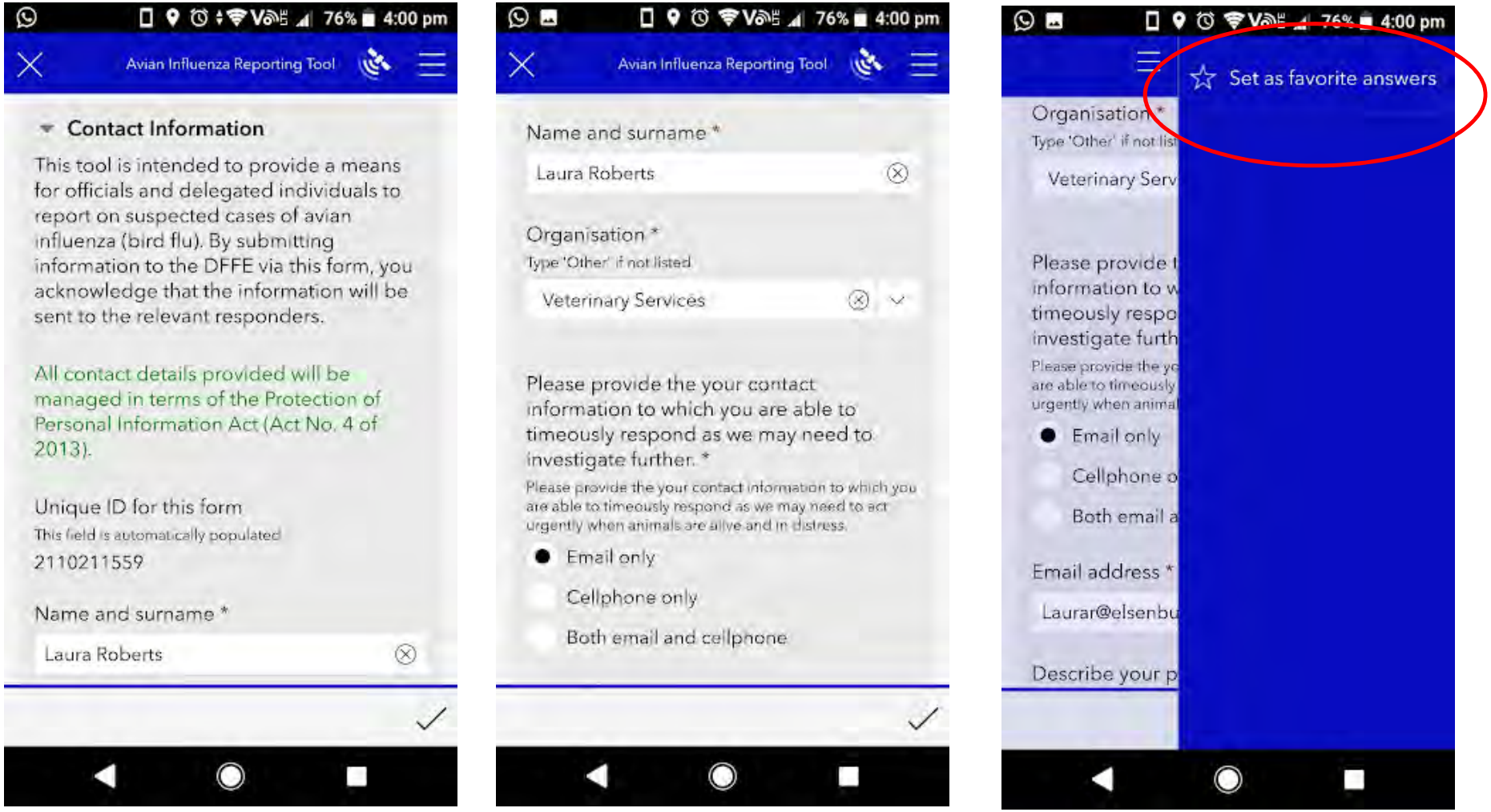


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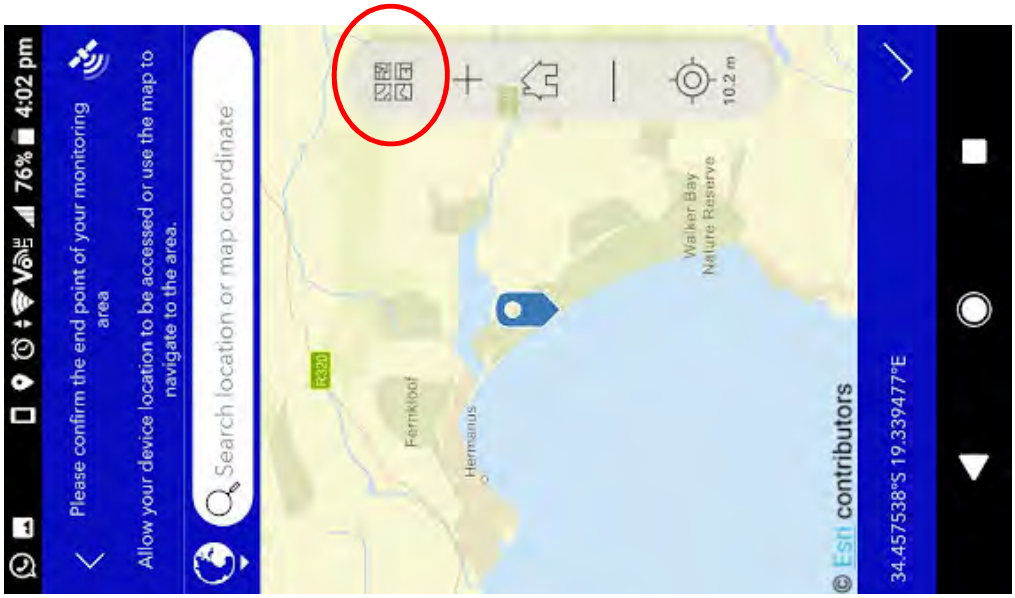


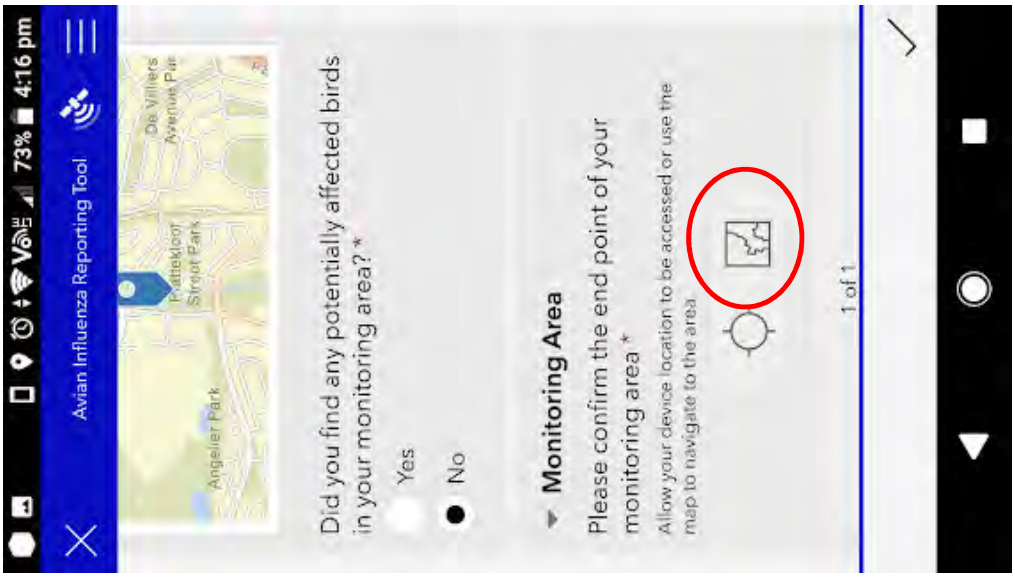
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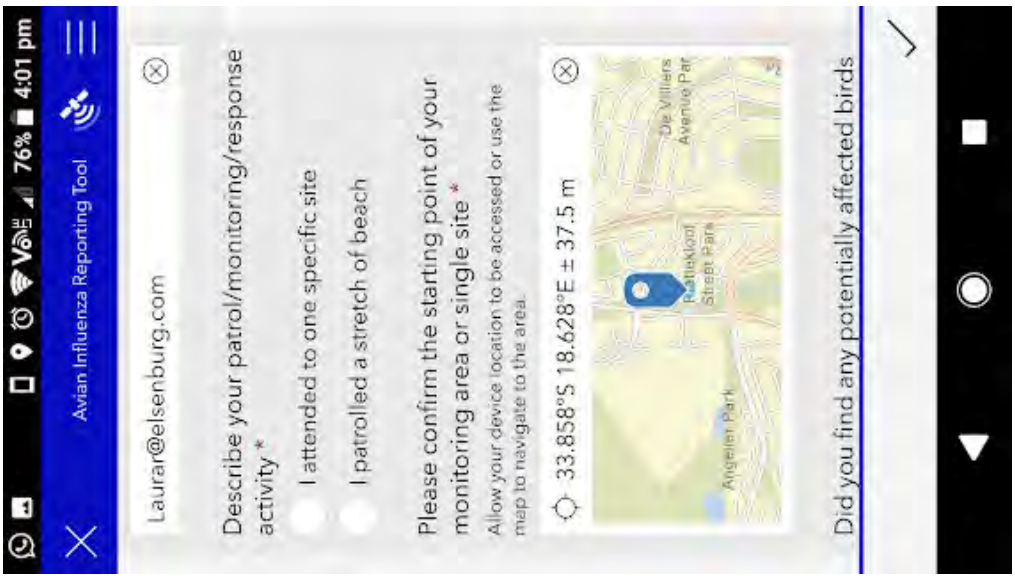


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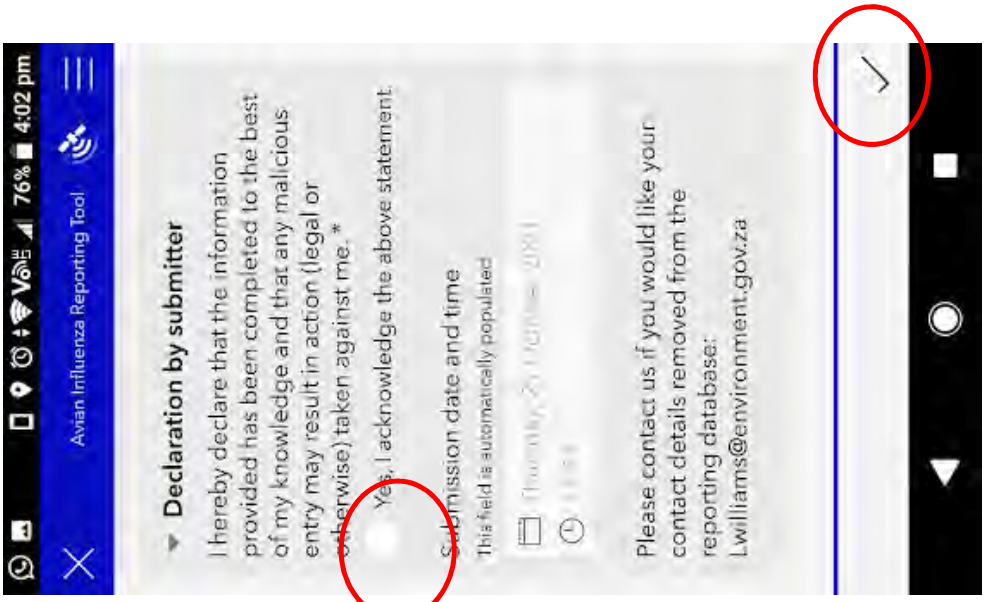
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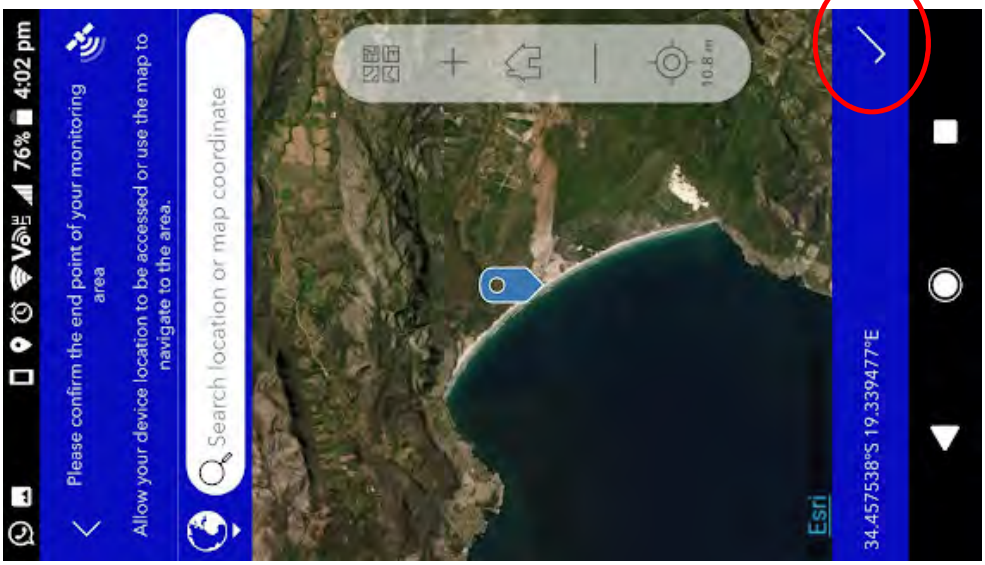


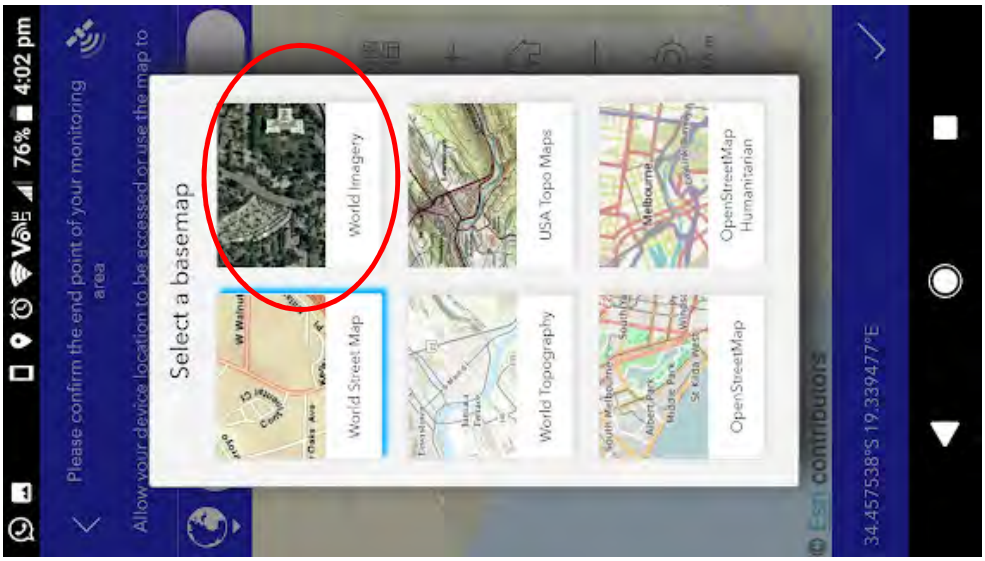




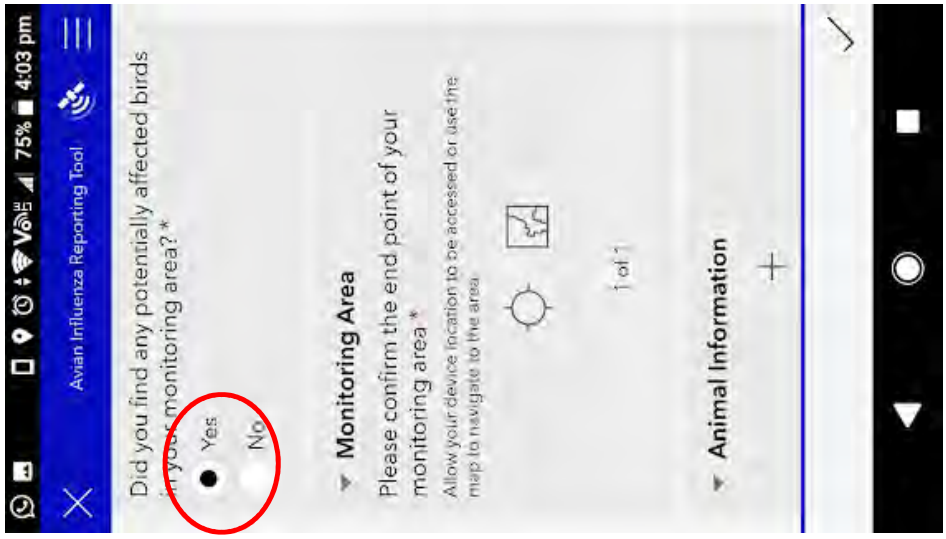
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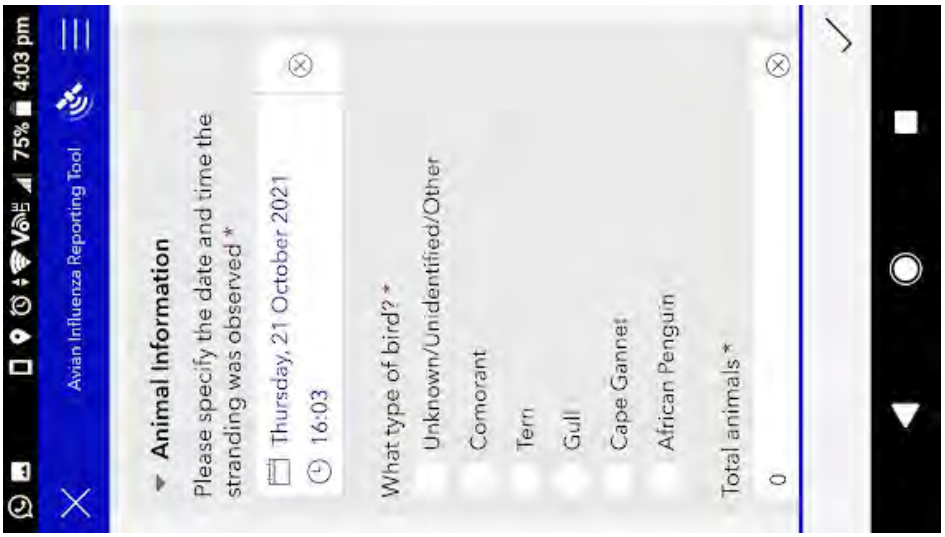






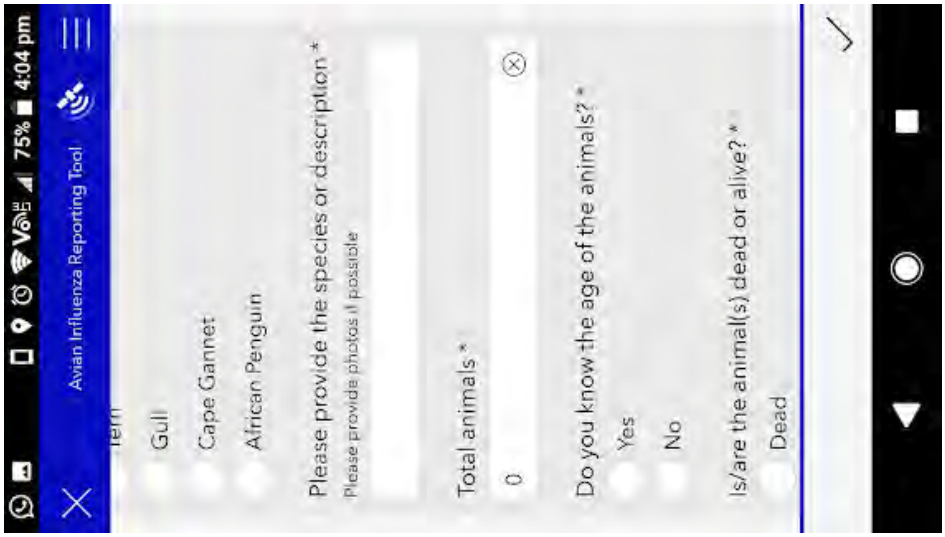
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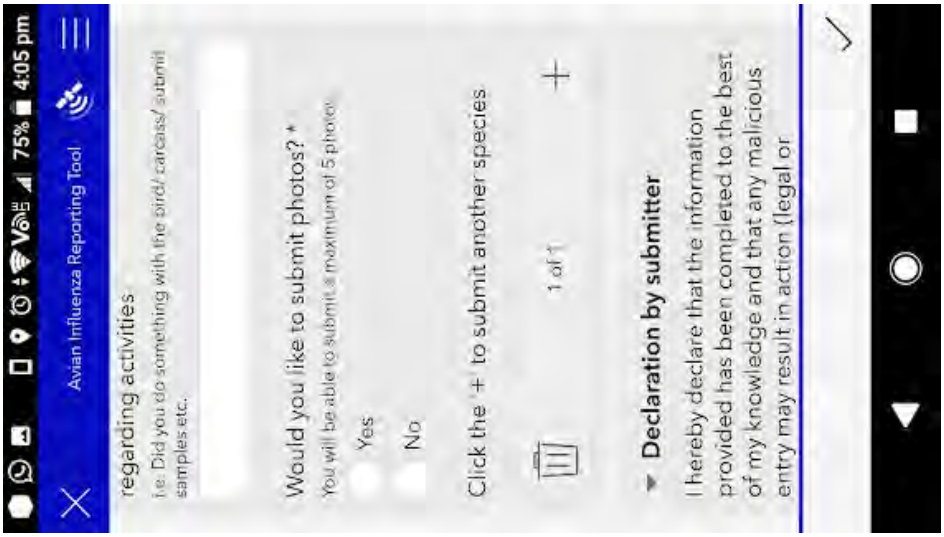
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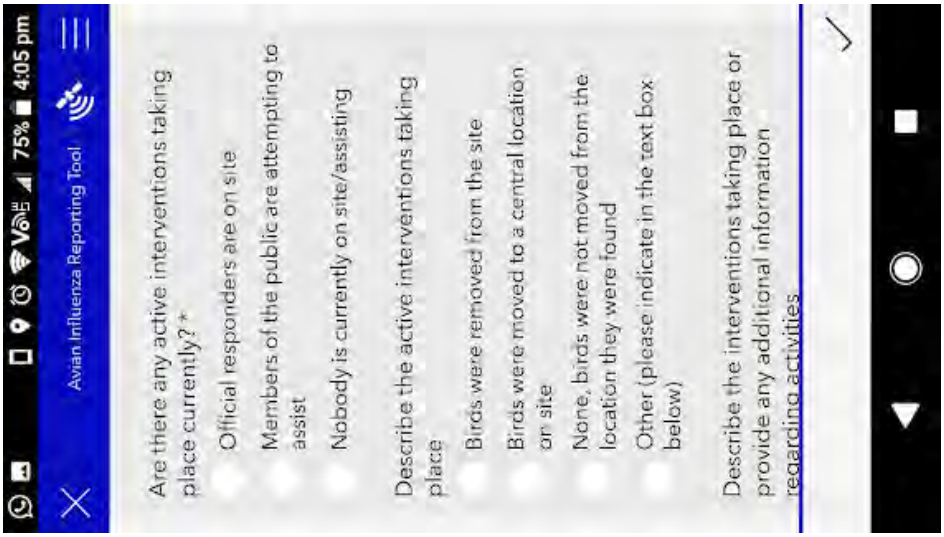


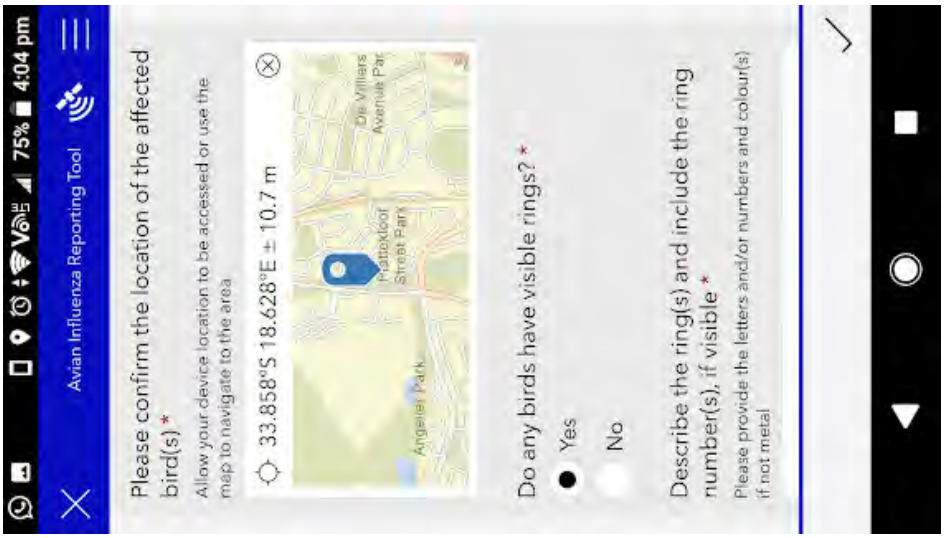




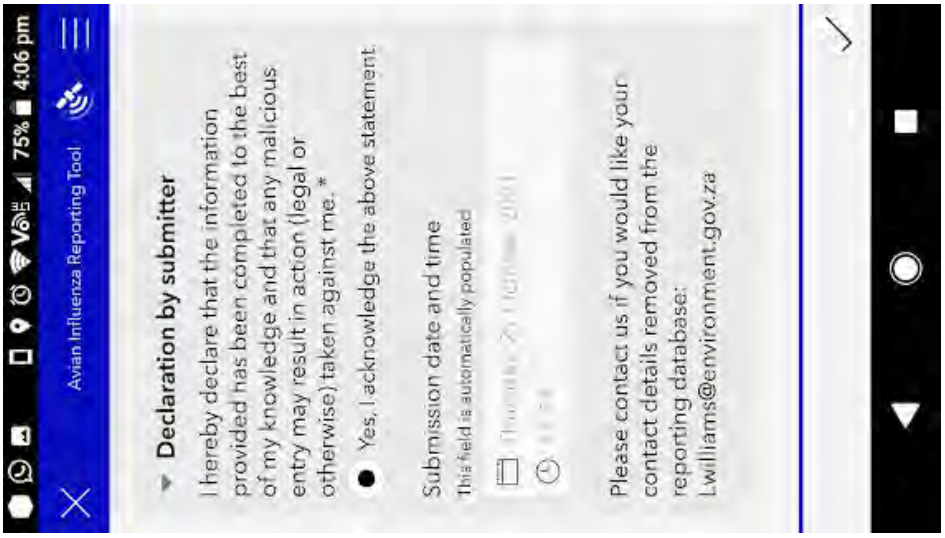
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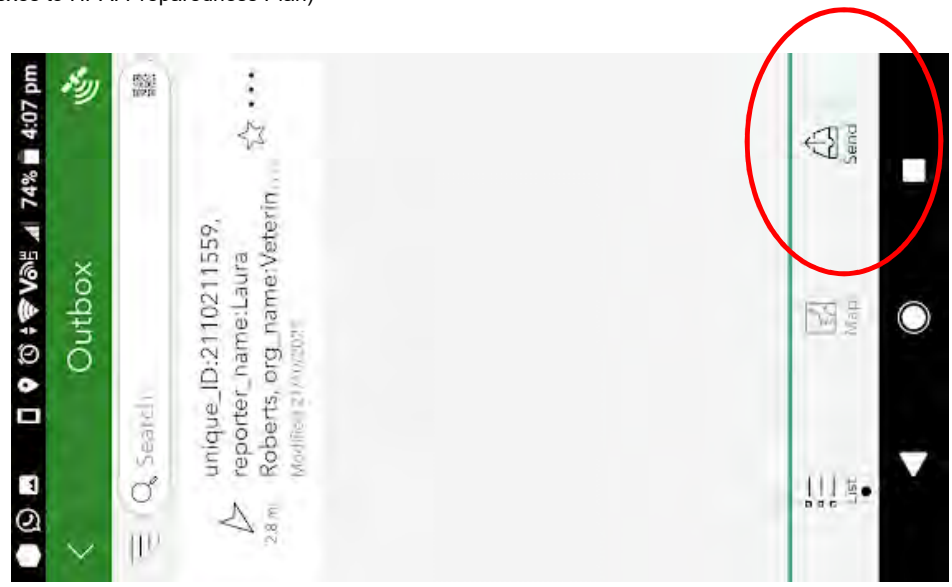


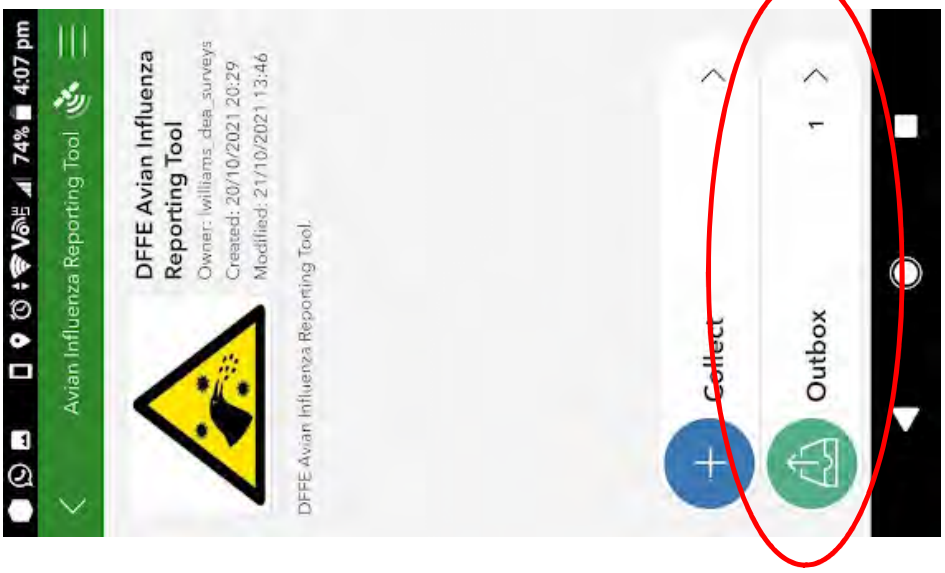
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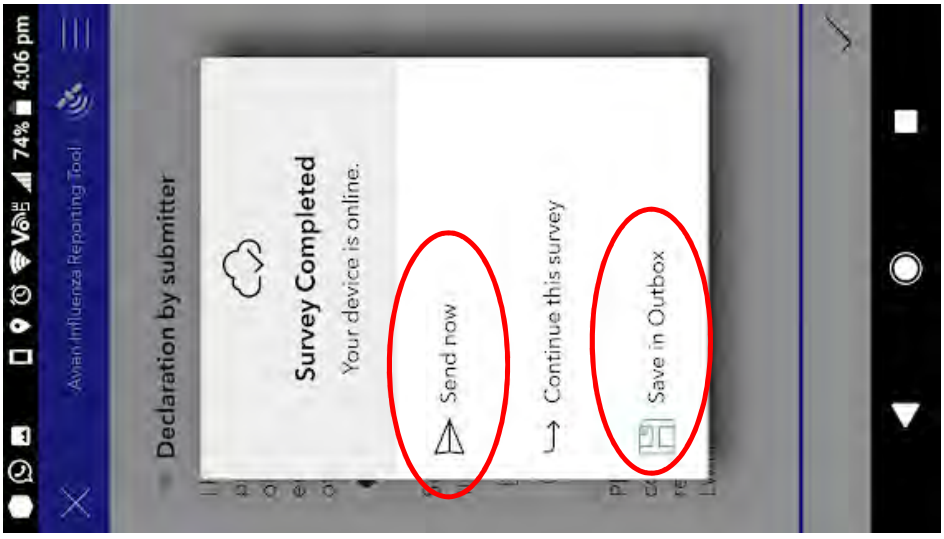




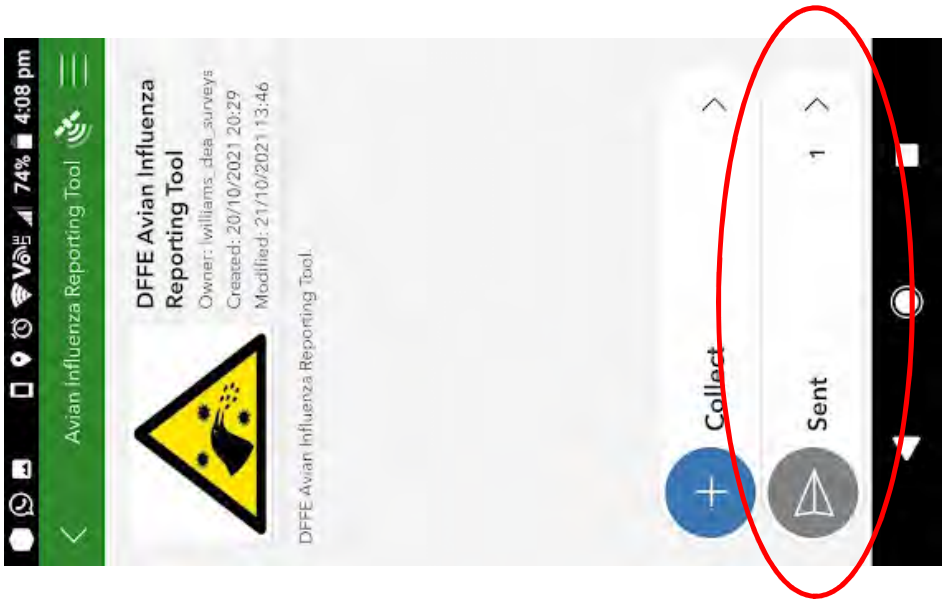
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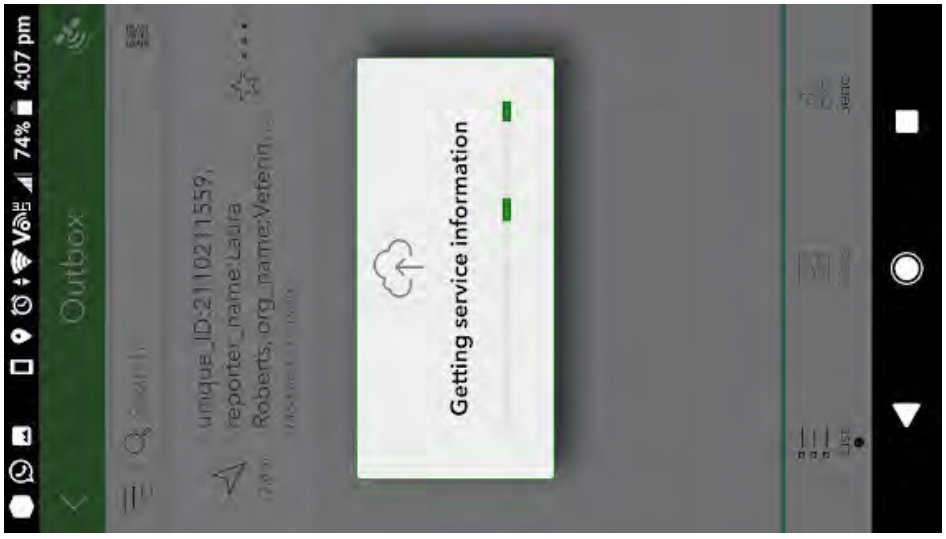






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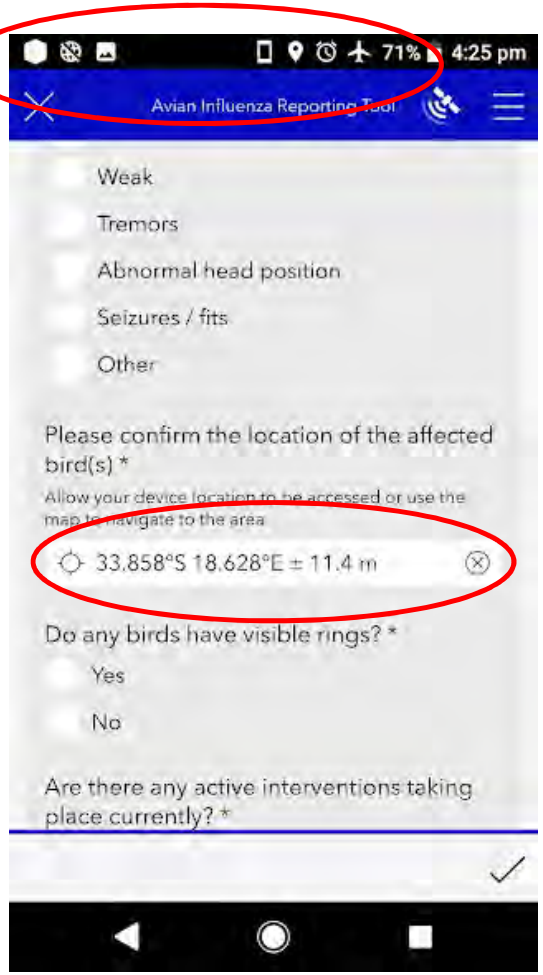




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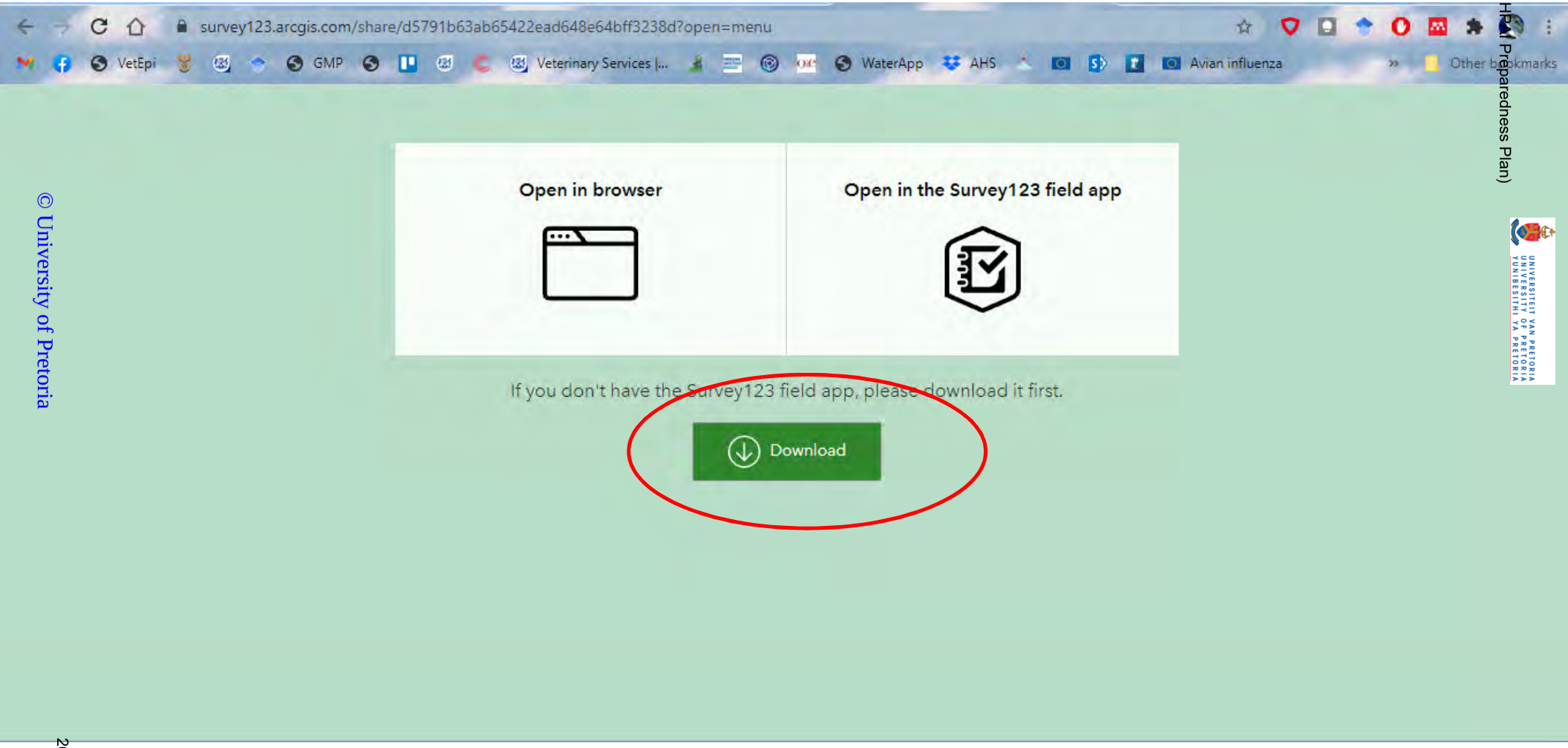
You can work offline

- Your GPS on your phone can work without internet but you won't see a map

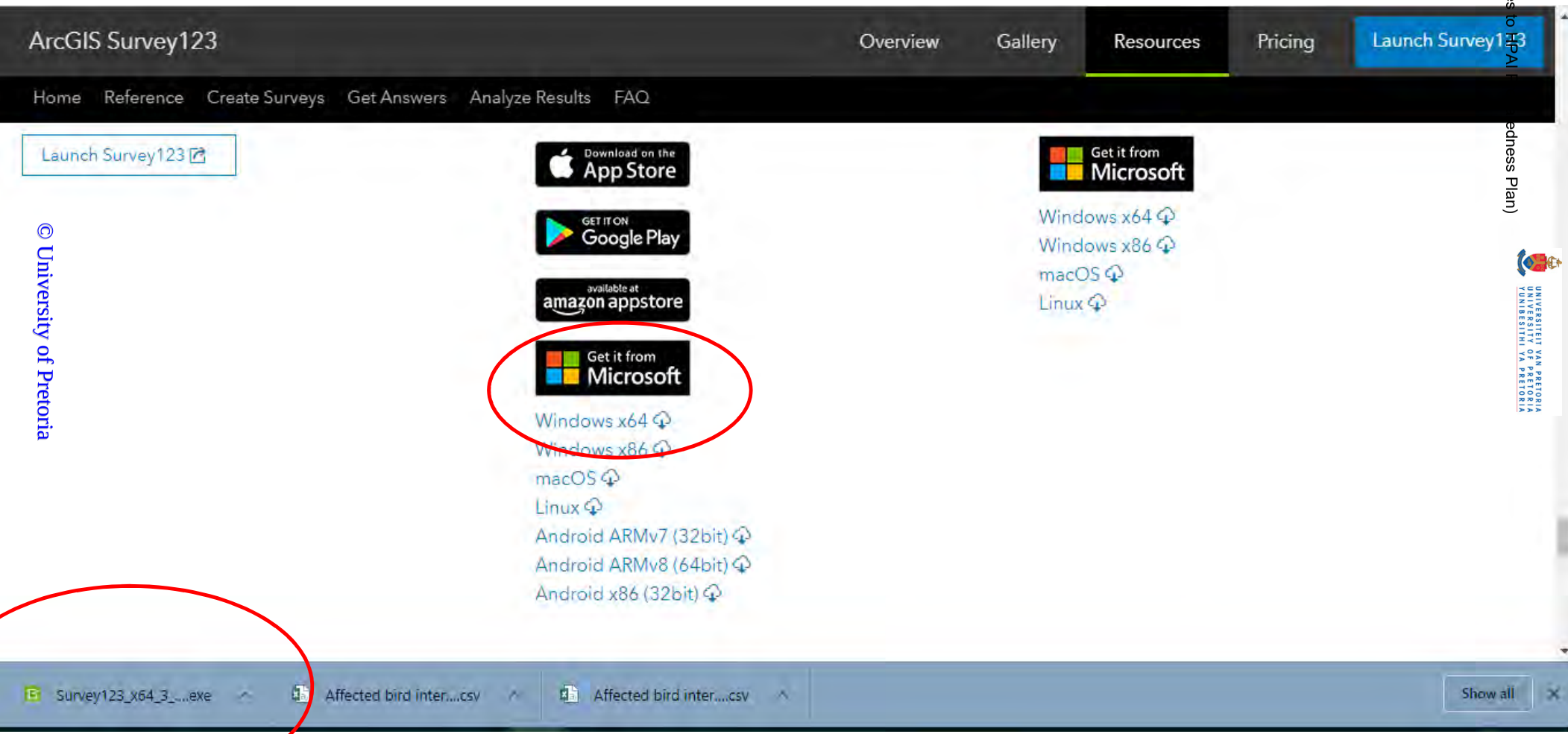


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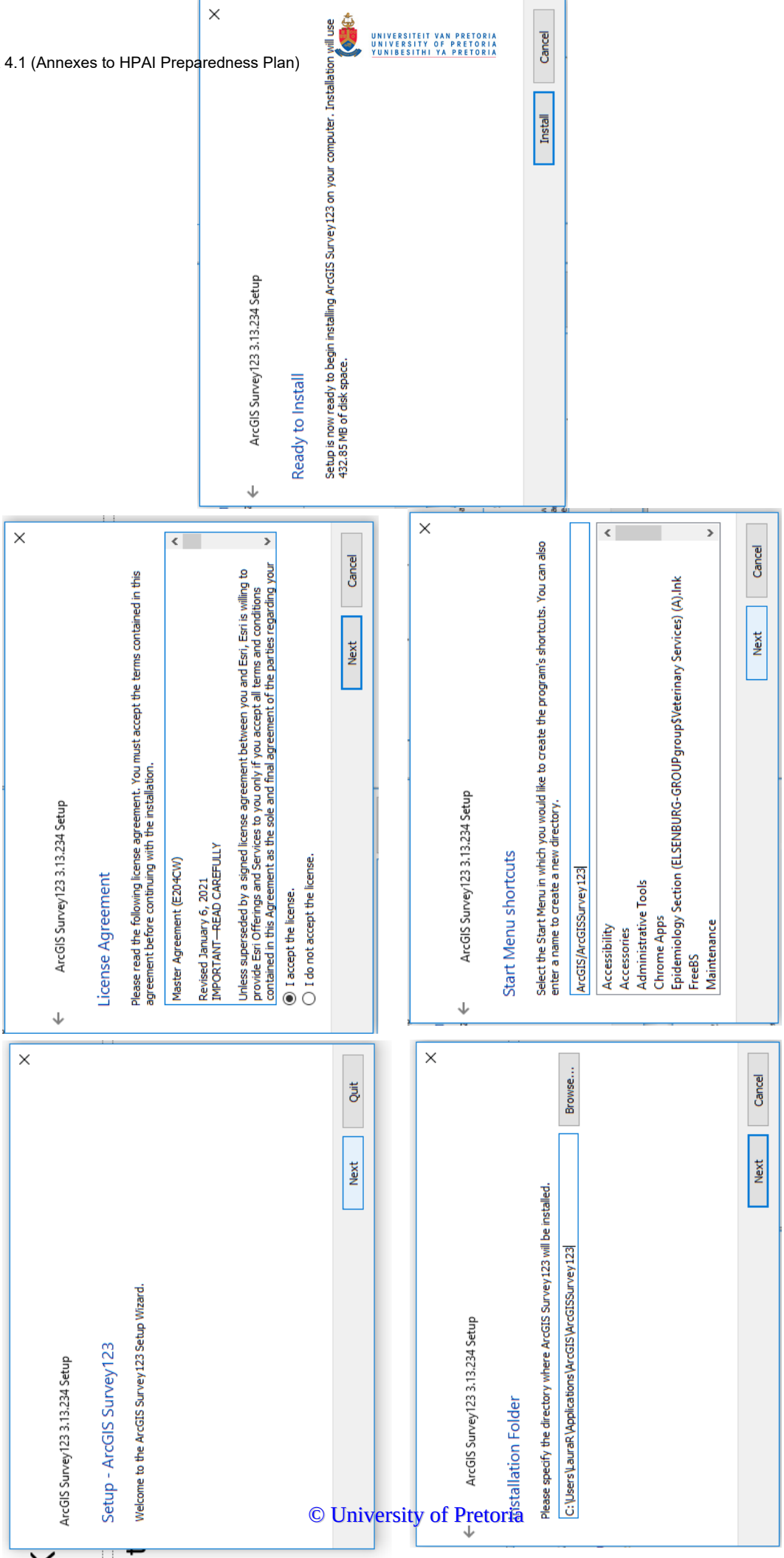
Diwnload the desktop (computer) app



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South African seabird disease outbreak preparedness plan

Annex 4.1.4: Mass Mortality Investigation

Person reporting

Name:_____

Email address: _____ Cell number:_____

Time and Place

Date of observations:_____

GPS coordinates (if available): S_____ E_____

Name of place and closest town/ suburb: _____

Land owner/ responsible person: _____

Cell number:_____ Email address: _____

Is the area accessible to the public? Yes/No

If No, what are the access requirements?_____

_____

Description of the incident

Who reported the incident? _____ Cell no. _____

What was the initial problem reported? _____

_____

_____

_____

When did the problem start? (date)_____

Estimation of morbidity/mortality:

Species	No. normal		No. dead		No. sick		% adults	
Select method:	*Counted*	*Estimated*	*Counted*	*Estimated*	*Counted*	*Estimated*	*Counted*	*Estimated*

Clinical signs (e.g. unusual behaviour or physical appearance) _____

_____

How are the affected birds distributed? (e.g. spread out? Grouped together?) _____

_____

_____

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Background/ environmental information

Land use and human activities _____

_____

Specific features of habitat/area (e.g. vegetation, soil type) _____

_____

_____

Environmental factors (for example, weather conditions surrounding outbreak, recent rainfall, sea conditions, recent local use of chemicals, changes in ground water levels, changes in domestic animal management)

_____

_____

_____

_____

Additional notes/ comments (other abnormalities or possible causes of illness?)

_____

_____

_____

Action Taken _____

_____

_____

_____

Samples

Number of carcasses by species collected and submitted

Species	Number of carcasses	Samples (type & number

Person/ laboratory submitted to_____

References

Bodenstein, B.L., 2016, Recording and submitting specimen history data, in Franson, J.C., Friend, M., Gibbs, S.E.J., and Wild, M.A., eds., Field manual of wildlife diseases: U.S. Geological Survey Techniques and Methods, book 15, chap. C3, 7 p., http://dx.doi.org/10.3133/tm15c3

Rose, K. Sick and Dead Bird Health Surveillance Sample Collection Protocol. Australian Registry of Wildlife Health

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South African seabird disease outbreak preparedness plan: May 2024

Annex 4.1.5: Template Media Statement

[Date]

Avian influenza detected in seabirds in the Western Cape

[What is the news?]

CapeNature and a number of conservation organisations, including the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB) are monitoring and taking action to contain the H5N1 strain of highly pathogenic avian influenza (HPAI), that is currently affecting birds in South Africa.

[Background]

The HPAI (H5N1) strain has been detected in sick and dead wild birds but, to date, the impacts are not as severe as those of the 2017/18 H5N8 strain, where a large number of wild birds succumbed to the flu. There is concern about the possible impact it may have on the Great White Pelican population, in the Western Cape. There are only an estimated 370 breeding pairs that reside in the province, and already 40 out of a group of 100 birds, have succumbed to the virus. Great White Pelicans are colonial breeders and are listed as vulnerable in the Western Cape, and currently only breed on Dassen Island.

[What must people do]

Members of the public, as well as like-minded stakeholders and organisations are therefore requested to be on the lookout for affected birds, and in the event of them encountering such birds, to report these sightings to their local state veterinarian (contact details available here)*. HPAI is a controlled disease, and this is therefore required by law. SANCCOB may be able to assist in a preliminary diagnosis, which will help with the wild seabird disease surveillance work, and thus also be informed about any potentially infected seabirds

“I hope that with continued collaboration, open communication, and swift responses, we can continue to tackle this disease effectively. We will endeavour to keep everyone informed of any changes as we become aware of them”, added Kevin Shaw, CapeNature Team Leader: Ecology.

[Allay fears/ reassure about action being taken/ stress role-players involved]

Fortunately, this strain of avian flu poses a low risk to humans, but people can transport the virus on their hands and clothes so should be cautious about handling sick and dead birds. CapeNature will continue to work in close collaboration with Veterinary Services, and other conservation partners, to monitor and combat the spread of the virus.

[Contact details]

Members of the public can report sick birds to SANCCOB on 021 557 6155 (office hours) and 078 638 3731 (after hours). State vet areas and contact details can be accessed here:

*https://www.google.com/maps/d/u/0/viewer?mid=1Mkck_eLOLvagqsMO6ufBUZX2DBw&ll=-32.65886130849003%2C20.98989000000003&z=7

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-ENDS-

MEDIA ENQUIRIES:

[Organisation]

Communications Officer

[Name]

[email address]

[Telephone number

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HIGH PATHOGENICITY (H5N1) AVIAN INFLUENZA IN THE WESTERN CAPE

Frequently Asked Questions (FAQs)

November 2021

1. What strain of Avian Influensa is causing the death of the sea birds?

The strain is assumed to be the same clade 2.3.4.4 H5N1 strain that has been present in South Africa since April this year. The testing to confirm is still in progress. The clade number is used to distinguish it from a different H5N1 strain found elsewhere in the world since 1997.

2. Where did the virus originate?

Eurasia. The H5N8 "grandparent" was found in Russia in 2016 and this H5N1 strain has developed from that one and other low pathogenicity viruses.

3. Is it a new virus?

Yes, it was first detected in Europe in 2020, though it is closely related to AI viruses that have been around for the last 5 years or so.

4. How does the virus spread and how quickly?

The virus is present in faeces and in the fluids in the mouth and nose of infected birds, as well as in tissues of dead birds. It's spread by direct contact with these fluids and tissues, and in aerosols (sneezing). It can also be transported on contaminated objects (vehicle tyres, shoes, clothes).

We don't know how quickly the virus spreads in wild birds. It probably differs between species and is a very complex question to investigate. In chickens, it can kill all in a house within 1-2 weeks, if allowed to spread, but the conditions are very different in the wild.

5. Which bird species have been affected?

In **Western Cape**, Lab-confirmed cases in:

Domestic chickens, turkeys, geese and ostriches

Cape cormorants

Cape gannets

White-breasted cormorants

African sacred ibis

African penguin (2)

Kelp gull

Hartlaub's gull

Great white pelican

Egyptian goose

Spur-winged-goose

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Blue crane

Yellow-billed duck

African fish eagle

Brown skua

6. **Are any of the birds species on the endangered list?**

   African penguins, Cape cormorants, Cape gannets

   Bank cormorants look similar to Cape cormorants and are very rare (only 5000 left) so we could miss the disease in this species.

7. **How do you contain the spread of the virus?**

   In wild birds, all we can do is try to reduce the amount of virus in the environment by disposing of carcasses and removing sick birds and euthaniszing them.

8. **Do you know at this stage how many birds have been killed by this virus?**

   As of 28 October, since approx. 12 October, just over 15 000 birds have been counted as sick or dead, along the Western Cape coastline, mostly Cape cormorants. We have no way of knowing how many died at sea or on beaches that people have not accessed or reported from.

9. **What will it mean to the species in the long term?**

   It's too early to tell, but given that the South African Cape cormorant breeding population was estimated to be 100 000 birds and the number lost equates to 15%, it is expected to be very damaging.

10. **Does this virus only kill wild sea birds or are poultry (farm animals) also at risk?**

    Yes, domestic poultry are at risk and it is probably more pathogenic to chickens. Twenty-three poultry and ostrich farms have already been affected this year in the Western Cape and 60 in South Africa. The latest was infected about 2 weeks ago.

11. **Is there a danger that the virus will spread to the human population or to other animals, e.g.seals, dogs?**

    There is a small chance that individual mammals could be infected (though not necessarily get sick), which is why people are asked not to touch carcasses and to wear gloves and masks, but it is not considered an imminent danger. No avian influenza has been known to gain the capability to spread from human to human.

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Annex 4.1.7: Personal protective equipment (PPE) requirements for handling infectious animals and material

The Centre for Disease Control (CDC) in the USA recommends the following PPE for people working with AI-infected poultry: "properly-fitted safety goggles, disposable gloves, boots, a NIOSH-certified respirator (e.g., N95), and disposable fluid-resistant coveralls."

The CDC also advises on how to "Safely remove PPE in sequence":

1. Remove and dispose of the apron, if worn;
2. Clean and disinfect boots;
3. Remove boots;
4. Remove and dispose of the coverall;
5. Remove and dispose of gloves;
6. Wash hands with soap and water;
7. Remove goggles and respirator;
8. Clean and disinfect reusable goggles and respirator;
9. Wash hands with soap and water again

European standard EN 14126 defines performance requirements and test methods for protective clothing against infective agents. Suitable clothing should be marked with the **biohazard pictogram** (Figure 1) and be **CE certified as Category III** to denote that it has passed the correct tests. CE 0120 is the number of the SGS company as a Notified Body to audit the certification.

Clothing types (Table 1) are classified according to their ability to protect against different media that could carry micro-organisms: gas, fluids at different pressures, and particles. A defined set of tests must be carried out on the fabric (e.g. ISO 16603 to test resistance to penetration by blood and body fluids and a range of pressures) (DuPont, 2014)

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Figure 1: The biohazard pictogram

Table 1: PPE clothing types

Type	Description	Standards
4-B	Protection against liquid aerosols (spray tight)	EN 14605:2005 + A1:2009
5-B	Protection against airborne solid particulates	EN 13982-1:2004 + A1:2010
6-B	**Limited protection against liquid chemicals (light spray)**	**EN 13034:2005 + A1:2009**

For working in a colony infected with avian influenza but where there will be no chance of spraying blood or faeces, Type 5&6 overalls should be sufficient. *For spraying blood/ faeces/ contaminated water, Type 4 may be necessary.* E.g Tyvek 500 suit

The standards of the respirators recommended by the US CDC (**NIOSH N95**) correspond to a mask that protects against 95% of airborne particles but is not oil-resistant. **EN149** is the European standard for a filtering half mask with exhalation valve, designed to protect against solids, water-based aerosols and oil-based aerosols. **FFP3 respirator masks** are, the most efficient in filtering out fine particles including viruses, mould spores, and asbestos as they filter out at least 98% of the airborne respirable particles.

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- https://www.cdc.gov/flu/avianflu/h5/worker-protection-ppe.htm
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- DuPont (2014) Clothing to prevent against infection. Luxembourg. Available online at: http://www.dupont.co.za/products-and-services/personal-protective-equipment/chemical-protective-garments-accessories/uses-and-applications/chemical-biological-hazard-protection.html
- http://multimedia.3m.com/mws/media/433598O/european-standards.pdf
- https://scandiagear.com/norms-and-standards/
- https://www.honeywellsafety.com/Europe/Product_Catalog/Product_Catalogues_-_Downloads.aspx

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Waste management Annexure A (v. Jul.2022)

Question	Answer	
Farm owner /Company/ Organisation		
Location (Farm & Site) name		
Area (Local municipality/ closest town)		
Postal address		
GPS coordinates (**disposal site**)	S	E
1. What quantity of material will be treated?		
2. What treatment method is proposed?		
3. Please provide detail in terms of duration of treatment planned, detailed methodology, and the outcome of the treatment process in terms of effectivity of rendering the virus inactive.		
4. Will the treatment method lead to the formation of leachate?		
5. Please provide a description of the proposed treatment area, and attach a photograph to the completed Annexure A.		
6. Has the proposed treatment Site been chosen to reduce the risk of ground and surface water contamination?		
7. Are there any water sources within 100 m of the proposed treatment Site? If so, please specify the source and		

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distance from the proposed treatment Site.	
8. Has wind direction with regards to odours been taken into account in the selection of the proposed treatment Site?	
9. Subsequent to the treatment process completed, what will be done with the remaining treated material?	
10. Will the State Veterinarian ensure the end product is free of any contamination?	
11. How will the Site be remediated after treatment has been completed?	
12. Please provide the detail of the person on Site managing the treatment process. Please provide details of the person's relevant experience, name, email and contact number.	
Declaration of accuracy of information provided: I ……..declare that the information provided in this report is in all respects factually true and correct. Signed at on this day of …….. SIGNATURE	

I, the State Veterinarian responsible for the area within which the proposed treatment Site resides, **support/does not support** (please circle) the proposed treatment option for this Site.

Signature Printed Name

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ANNEXURE B:
AVIAN INFLUENZA – REQUEST FOR TREATMENT THAT MAY IMPACT ON AIR QUALITY

This document is to be completed when the proposed treatment method **(Annexure A, Question Number 3)** is "**Incineration or other process that may impact on air quality**". This document is required to be submitted, together with **Annexure A**, to the **LEAD AUTHORITY (Directorate: Waste Management, DEA&DP)**, if applicable.

Note: The request for treatment will be addressed on a **"case-by-case"** basis, by the relevant Municipality.

DETAILS OF TREATMENT PROCESS		
1. Municipal Area		
2. Name of Enterprise		
3. Industry Type? Nature of Trade		
4. Telephone Number		
5. Details of proposed treatment process		
6. Has the Enterprise engaged with Disaster Management at the District Municipality / Metropolitan Municipality?	**Yes**	**No**
7. If yes, provide outcome of discussion. **8. If no, confirm that relevant District or Metropolitan Municipality will be engaged.**		

Declaration

Declaration of accuracy of information provided:

I, , ...declare that the information provided in this report is in all respects factually true and correct.

Signed at on this day of .....

SIGNATURE

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APPROVAL

Any deviation from **Annexure B** must be communicated to the relevant **Disaster Management official at the Municipality** and / or the **Municipal Air Quality Officer** and **Provincial Air Quality Officer**.

I, the State Veterinarian responsible for the area within which the proposed treatment Site resides, **support/does not support** (please circle) the proposed treatment option for this Site.

Signature Printed Name

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Annex 4.1.9

Euthanasia of seabirds

Euthanasia (from the Greek, meaning "good death") refers to ending an animal's life to reduce suffering. When animals must be euthanised, using the correct methods will reduce suffering and make this unpleasant task simpler.

In many cases, sick or injured seabirds can be transported to a veterinary clinic or rehabilitation centre where a veterinarian can examine the bird and decide to either treat or euthanise it. Where this is not possible or where transport would cause unnecessary suffering or disease transmission, euthanasia may be required on-site.

Many seabirds are legally protected, some are threatened or endangered species, and most seabird colonies are in protected areas. Before considering euthanising animals, permission must be given by the conservation authority responsible for the area. In some cases, any form of handling or restraint or euthanasia will require a TOPS (Threatened Or Endangered Species) permit.

Animals that are being collected, transported or handled for euthanasia must always be treated gently and with respect, to reduce suffering wherever possible.

Standard euthanasia policy

If any bird has a severe injury or disease which is untreatable or prevents rehabilitation to a level where it can survive productively in the wild, it should be euthanised humanely.

Factors to consider

- ***Prevention of unnecessary suffering:*** For individuals that have untreatable conditions which will result in severe suffering or other welfare concerns, euthanasia is considered the most humane option.
- ***Caseload and resources available:*** Resources such as the availability of suitable facilities and equipment for rehabilitation, suitable space for individual species and staff capacity may be limiting factors in ensuring that animals receive an appropriate level of care. These limitations need to be taken into consideration when making decisions about whether animals should be treated and selecting animals to be treated first.
- ***Conservation status of species:*** If it is not possible to provide care for every animal, the conservation status of seabirds can be used as a tool during the triage process, e.g., species of greater concern should receive priority, or breeding adults of species of conservation value should receive priority care over juveniles.
- ***Infectious diseases:*** Individuals carrying an infectious disease that may pose a risk to the health of other animals undergoing rehabilitation may need to be euthanised to prevent the risk of spreading the disease.
- ***Prolonged time in captivity:*** Animals needing prolonged care may develop secondary health problems related to captivity, which will influence the outcome of rehabilitation. Additionally, extended time in captivity may influence the behaviour of the animal once it is released so that it cannot contribute to the breeding population.

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Chemical euthanasia

Chemical euthanasia, if performed correctly, is the preferred method of euthanasia in most cases. It involves the injection of an overdose of an anaesthetic agent which causes the patient to become unconscious and then stops the heart, breathing and brain activity, leading to death. Sodium pentobarbitone (200mg per kg) is commonly the drug of choice.

In most cases, only a veterinarian, registered with the South African Veterinary Council, may euthanise an animal by injection of a euthanising medication. The procurement, transport and storage of anaesthetics are highly regulated and cannot be done by unregistered people.

Chemical euthanasia procedure

- Chemical euthanasia can only be performed by a person who is legally registered to do so, usually a veterinarian.
- Euthanasia should preferably be done under sedation or general anaesthesia unless this is impossible, or in cases when quick euthanasia without sedation or anaesthetic would reduce animal suffering.
- Euthanasia should be done by intravenous injection whenever possible
- If an intravenous injection is not possible, intrahepatic, intracardiac or oral administration of the drug are other options. An injection into the foramen magnum at the base of the skull is also effective. In these cases, sedation or general anaesthesia is recommended to reduce animal suffering, pain and distress.
- The animal should be properly restrained throughout the process.
- The heartbeat and respiration must be checked with a stethoscope to confirm death.

Advantages

Quick, Painless, Bloodless

Disadvantages

Needs highly controlled medication; needs a veterinary professional; the carcass is toxic after death and can poison other animals.

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Non-chemical euthanasia

When a vet is not available and transport to a vet is not possible, euthanasia can be performed through traumatic means. In these cases, every effort should be made to reduce suffering.

There are many methods of quickly killing a bird and the method that is chosen will depend on the skill of the handler, species, equipment, facilities and biosecurity (disease control) requirements.

Cervical dislocation

This method refers to breaking the spinal cord. It requires some training and strength and is only possible in smaller birds but leads to a relatively quick and bloodless death, which is recommended in cases of infectious diseases.

Species

Most birds weighing less than 1kg, for example waders, terns, small gulls, and small cormorants. It is possible in Cape Cormorants but not for gannets, penguins, or Kelp Gulls.

Method

Video: https://photos.app.goo.gl/V9SzANKdXRADQpmr5

Restrain the bird's body with your less dominant hand and hold the head in your dominant hand. Keep the head in the palm of the hand with the neck between the index and middle fingers. Bend the neck so that the head points upwards at 90 degrees to the neck and twist it slightly to one side (Fig. 1 A). Pull the neck away from the body, steadily and firmly. For birds over 500g, this requires some effort, pushing downwards with the whole arm. The aim is to dislocate the neck vertebrae where they attach to the head and to break the spinal column. When it is done correctly a soft, boneless area can be felt behind the head (Fig 1B). Once this has happened, the bird is paralysed and will die within a minute. Check that the heart has stopped to confirm successful euthanasia.

A.  B. 

Figure 1: A: position adopted for cervical dislocation of a Cape Cormorant. B: site to feel for successful cervical dislocation

Advantages

Bloodless, relatively quick, easy to learn, requires no special equipment

Disadvantages

Requires practice and training, not painless, not suitable for birds larger than a Cape Cormorant

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Piercing the brain or spinal cord

A strong, sharp knife is used to pierce the skull or to cut the spinal cord where it enters the skull, paralysing and killing the animal.

Method

In most birds, it is possible to force the tip of a strong knife through the top of the skull. The bird is restrained with the head on a solid surface or the ground. A strong, sharp knife is held vertically, with the tip in contact with the skull, near the back of the head, and then firmly pushed through the skull and into the brain. The same method can be used at the base of the skull to sever the spinal cord where it enters the skull.

Advantages

Mostly bloodless, usually quick

Disadvantages

Restraint is difficult without an assistant; requires a good understanding of the relevant anatomy, practice and training; requires the right equipment.

Traumatic stunning

This refers to stunning the bird into unconsciousness by striking the head. If done properly, a sharp knock to the head will quickly stun or kill a bird. A stunned bird can then be killed by exsanguination (bleeding out) or decapitation or another method.

Method

Several methods can be used. But the preferred method uses a captive bolt device specifically designed for euthanising small animals. This device uses a spring-loaded bolt (or one powered by a blank charge) to stun an animal. Striking the head with a solid, heavy instrument like a hammer is also possible, or striking the bird's head against a solid object. If done incorrectly, this method can cause excessive suffering if the bird is wounded but not killed or stunned.

Advantages

Quick

Disadvantages

If done ineffectively, it can cause severe pain and suffering; ideally, it requires special equipment; to be done successfully it requires training and practice; not necessarily blood free

Decapitation

Decapitation refers to removing the head.

Method

The entire head is cut off as quickly as possible using a heavy knife, machete or axe. The bird is restrained against a chopping block, or similar object, so that the neck is exposed, and the head is removed.

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Advantages
Reasonably quick

Disadvantages
Causes a mess and poor biosecurity; can be dangerous for the people involved; requires training and practice

Exsanguination
Exsanguination refers to any method that causes bleeding on a scale that leads to death. To achieve humane euthanasia, this is usually done by cutting the large blood vessels in the neck.

Method
A sharp knife is used to cut the neck under the spine, below the jaw, and close to the head. Care must be taken to cut both the major arteries (carotid arteries) and veins (jugular veins) on both sides of the neck.

Advantages
More controlled than decapitation; can be done with a small knife

Disadvantages
Causes a mess and, poor biosecurity; not as quick as other methods; if the knife is not sharp enough it can be difficult to achieve and lead to excessive animal suffering.

Conclusion
No method of euthanasia is possible without causing some level of discomfort, stress or pain to the patient. However, when euthanasia is carried out in a way that reduces the severity of or time spent suffering, it is a means to improve animal welfare and provide a better death.

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PROTOCOL FOR THE MANAGEMENT OF HIGH PATHOGENICITY AVIAN INFLUENZA IN SEABIRDS

Updated: March 2024

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1. PURPOSE

The purpose of these protocols is:

To provide specific guidelines for responding to high pathogenicity avian influenza (HPAI) outbreaks in seabirds, in addition to the information provided in the South African seabird disease outbreak preparedness plan (hereafter referred to as the *Preparedness Plan*), in order to:

a) Aid early detection of high pathogenicity avian influenza (HPAI) outbreaks in seabirds and to assist with quantifying their effects
b) Provide guidance in the management and handling of seabirds susceptible to and infected with HPAI.
c) Implement biosecurity measures to mitigate the spread of and mortality caused by the virus.
d) Reduce the risk to human health and domestic poultry.

2. BACKGROUND

High pathogenicity avian influenza (HPAI) has in recent years caused unprecedented mortality of wild birds worldwide, including in South Africa, with threats to population levels for some species already under multiple anthropogenic pressures. HPAI is a disease caused by a virus and is highly contagious in birds. It can be spread long-distance, across and between continents, by wild water birds.

Since 2017, HPAI has caused millions of deaths in commercial chickens and severe damage to the ostrich industry in South Africa. To date, over 30,000 South African seabirds have been affected, mainly Cape Cormorants, Swift Terns, and African Penguins.

In October 2023, HPAI (H5N1) was detected in seabirds and marine mammals on the island of South Georgia and it was detected on Antarctica in February 2024. The risk to colony breeding seabirds on the Prince Edward Islands has therefore also increased.

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3. HPAI VIRUS TRANSMISSION AND SPREAD

Avian influenza viruses are transmitted through guano and discharges from the mouth, nostrils, and eyes of infected birds. Any bird carrying HPAI virus can spread the disease to other birds through direct contact, or through respiratory droplets, when in close proximity. The virus is therefore able to spread rapidly between birds that spend extended periods close together, as in dense seabird colonies. Dead birds will also have virus present in tissues and organs and can be source for other animals, especially scavengers.

The virus can also be spread through contaminated water or fomites (contaminated objects) which can include equipment, vehicles or humans with contaminated clothing or hands.

The virus survives particularly well (up to months) in cool, dark conditions and in damp areas and organic matter such as guano or mud. High salinity conditions and extremes in pH shorten virus survival time.

4. RISK TO PEOPLE

Between January 2021 and 14 July 2023, there were at least 16 detections of HPAI (H5N1), of the newer strain (Clade 2.3.4.4b), in humans worldwide and this strain is believed to have caused deaths in thousands of wild mammals. The risk to humans is still low and no mammal cases have been detected in South Africa, but the risk of human infection has increased from previous strains.

People slaughtering poultry and those closely associated with infected birds are at highest risk. An infection could occur through contact with infected body fluids, such as aerosol or droplets, in the eyes, nose or mouth, especially if carcasses are opened. People need to take precautions to not only prevent spreading the virus to other birds but also to protect themselves (see Section 12). Strict health and safety measures should be employed for those handling infected birds and materials. It is also advised that seasonal flu vaccinations are received, to reduce the chance of avian and human flu viruses combining.

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Although the risk for human infection with HPAI is generally considered low, people who develop flu-like symptoms within 10 days of being in contact with a sick bird should seek medical attention and inform the healthcare professional that they have had contact with sick or dead birds and ask them to alert the National Institute for Communicable Diseases (NICD) on the clinician Hotline number (0800 212 552).

5. CLINICAL SIGNS OF HPAI IN BIRDS

Infections with HPAI in seabirds have mainly presented as neurological signs varying from depression and unusual docility or tameness, though subtle twitching, and tremors to obvious abnormalities such as walking or swimming in circles, whole-body tremors and seizures or fits occur in severe cases. Some signs of respiratory infections, including nasal and ocular discharge and swollen sinuses have been seen and some birds have eyes that appear cloudy, due to corneal oedema. Affected Northern Gannets in Europe were observed to have dark irises. A video of clinical signs can be found by following this link:
https://photos.app.goo.gl/bHh7oH4HLbpFrcBZ7

Marine mammals that are seen behaving unusually or showing difficulty breathing should also be reported.

6. REPORTING

Refer to Section 6.1 of the Preparedness Plan for monitoring and reporting of seabird mortality and illness.
Laboratory testing is needed to confirm that HPAI virus is present in a population, though there can be a strong suspicion based on observation of typical clinical signs and a drastic increase in mortality rate. Suspected cases of HPAI must be reported to the local state veterinarian, via the managing authority, as it is a controlled disease under the Animal Diseases Act. Contact details are available on the website of the national Directorate Animal Health.

If you see more than 5 dead birds in one area or birds showing suspicious symptoms, at least record:

a) Date found
b) Exact location found (GPS coordinates or street address)

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c) Species
d) Number of birds affected
e) Age category (chick/ juvenile/ adult)
f) If the bird was found alive or dead
g) If alive, what symptoms was it showing?
h) If dead, what state of decay was it found in?
i) What was done with the bird?
j) Name and contact details if further information is required
k) Photographs of dead birds should be taken from above and below, with the wings stretched out and tail spread as well as a side view of the head. These are especially important if the species has not been identified with certainty

Preferably, reports dead birds or birds showing HPAI symptoms should be done directly on the Department of Forestry, Fisheries and the Environment's (DFFE) Seabird Avian Influenza Reporting Tool:

- The Survey123 app can be downloaded from the Google Play Store or the Apple App Store and then the project can be loaded using the QR code below.



- Alternatively, you can follow this link in a web browser **https://arcg.is/1585011** and open the app, to link to the project.
- **Please select "Continue without signing in", on the Survey123 login page.**
- The reported data can be viewed on a dashboard, web map, and database here. Data can be downloaded as a CSV file (under Options) from the web map page.
- More detailed instructions are available in Annex 4.1.3.

If reporting in this way is impossible, a reporting template is available as Annex 4.1.3 to the Preparedness Plan and can be completed in Microsoft Excel or printed and completed on paper.

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7. SPECIMEN COLLECTION FOR LABORATORY TESTING

7.1. Introduction

Confirmation of HPAI infection is done through specific laboratory tests, most commonly a PCR test to detect the RNA (genetic material) of the HPAI virus. Genetic study of viruses detected can also assist with understanding possible origins and routes of virus transmission.

Testing for avian influenza virus in South Africa can only be done in laboratories that are approved by the national Directorate Animal Health of DALRRD. Surveillance done for research purposes also requires approval from this directorate, under Section 20 of the Animal Diseases Act No. 35 of 1984.

7.2. Sampling live birds

To confirm if live birds are affected by the HPAI virus, oral/ pharyngeal and/or tracheal and cloacal swabs can be collected. If the bird cannot be handled for sampling, fresh guano can be sampled after the bird has moved away. Insert the swab into the faecal component and avoid the white urate portion.

7.3. Sampling dead animals

Brain swabs should be collected from dead animals, along with oral/tracheal and cloacal swabs. Sampling of the lung is important if neurological clinical signs are not dominant but HPAI is still suspected.

If a post-mortem examination and/ or sampling cannot be performed immediately, complete carcasses can be refrigerated for future sampling (within approximately 3 days). The carcasses should be properly packaged in three plastic bags, labelled and the outer packaging disinfected.

7.4. Sample transport

All samples should be kept cool but not frozen (in a fridge and transported in a cooler box with an ice brick) and protected from UV rays (i.e. direct sunlight). Where immediate transport to a laboratory is not possible (e.g. due to long voyage times), specialised transport media like RNA*later* or DNA/RNA Shield must be used to allow freezing of samples below -20°C and long-term storage.

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7.5. Sample records

Please keep careful records of samples taken so that test results can be matched to the information about the sampled animal. Information can be added in the App, but should also kept in a sample list (template supplied in Annex 4.1.3.

7.6. Sample numbers

Try to sample at least five live and five dead individuals of each species, once the mortality rate and clinical signs have been discussed and concluded to be consistent with HPAI.

8. MANAGEMENT OF SICK BIRDS

Refer to section 7.4.1 of the Disease preparedness plan

Sick birds are a source of HPAI virus for other birds and will be suffering. Removal of these birds may help to slow spread of HPAI. However, the effect is unlikely to be significant as not all sick birds will be observed and birds will begin shedding virus before they appear ill.

Decisions about managing sick birds are similar to carcass removal: related to available resources and colony disturbance. The highly infectious nature of HPAI virus must also be considered when considering transport and rehabilitation.

9. MAKING A DECISION ABOUT CARCASS REMOVAL

Please refer to section 7.4.1 of the Preparedness Plan

The carcasses of birds that have died from HPAI are potential sources of HPAI virus which can infect other birds, especially scavengers. Removing these carcasses should reduce the quantity of virus in the environment and reduce the risk of infection in other birds. Removing carcasses from targeted areas such as freshwater bathing and/or drinking sites or high-traffic areas (e.g. pathways) may be especially effective. Frequent collection of fresh carcasses is ideal, as carcasses will be most infectious soon after the bird dies. In severe outbreaks, large numbers of dead birds may be found on public beaches where they may also pose a public nuisance or a public health risk.

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However, the contribution of carcasses to the overall amount of virus in the environment may be of far less importance that the amount shed by infected birds and the cost and harmful effects of carcass collection should be weighed against the possible benefits.

If carcasses need to be removed, this is the responsibility of the managing authority, which is sometimes a local municipality. Accurate records must be kept of the numbers of carcasses collected, the date, time and location collected, species, ages, and transport and disposal methods (See section 6).

10. CARCASS DISPOSAL

Refer to Section 7.4.1 of the Preparedness Plan for carcass disposal options

Carcasses with a **collective mass of less than 10kg** can be buried, if suitable ground is available. It is best to bury as close as possible to the site where the carcasses were found, but above the ocean's high tide mark and at least a metre deep (the sand must be a metre deep when replaced on top). Burial sites must be at least 100m from freshwater sources and care must be taken that contamination of groundwater should not occur.

Composting of HPAIV-infected carcasses

Composting performed correctly will cause the carcass temperature to rise enough to inactivate the virus but it requires adequate technical knowledge and plant material to be added to the carcasses. Consultation with an expert is advised. The material should reach a temperature of 55°C for 3 consecutive days during composting to destroy the virus. Access to the carcasses by scavengers and the dispersal of feathers during the process must be prevented.

Transport of carcasses

Transport of potentially infected carcasses is strongly discouraged, as there is a risk of spreading the virus. If transport is necessary, carcasses must be sealed in three layers of plastic or in a leak-proof, feather-proof container and any vehicle used must be cleaned and disinfected afterwards.

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11. BIOSECURITY GUIDELINES

Any person, vehicle, or piece of equipment that touches an infected bird, contaminated object, or environment can transmit the HPAI virus to a healthy bird. Clothing, equipment, and skin that has been in contact with or possibly contaminated by an infected bird or organic matter must be cleaned of dirt and any organic matter and disinfected before it touches another bird or enters a colony.

Vehicles should also be cleaned and disinfected before moving from one part of a colony to another and between colonies, especially the foot wells, wheels, and undercarriage of road vehicles. Dirty equipment and clothing should be cleaned before loading for transport, or packaged to prevent contamination of the vehicle.

Additionally, people who have worked with or handled seabirds in their colonies when HPAI is suspected to be circulating in wild seabird populations should not visit any domestic poultry farm or another seabird colony within the following three days and should not wear any of the same shoes or clothing unless they have been thoroughly cleaned and disinfected.

Special care should also be taken with other captive birds or birds undergoing rehabilitation. People who have visited colonies should not enter a rehabilitation centre without at least a full change of clothes (including shoes) and disinfection of all exposed skin.

11.1 Suggested clothing and personal protective equipment (PPE) for working in seabird colonies

Biosecurity and PPE recommendations have been matched to risk level for different activities and disease situations (see section 12). They are designed both to protect individuals and prevent spread between sites and species. Individuals may choose to adopt a more precautionary approach, but this is considered the minimum. PPE categories increase as the probability of HPAI virus exposure increases (highest for handling infected animals) and the risk of human infection rises (highest after there have been detections in other mammal species).

Category A PPE (for lowest HPAI risk/ normal activities)

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- Overalls/ rain suit/ oilskin jacket and trousers that keep clothes and skin clean and dry underneath
- Disposable gloves or hands/ gloves cleaned and sanitized between individuals.
- Footwear and outer wear to be cleaned and disinfected between sites/ areas of high wildlife density with appropriate disinfectant.

Clothing should be dedicated to colony work and should ideally be waterproof but should be as impermeable as possible and should have minimal folds and seams and no Velcro that will catch dirt and guano. It should be able to be sprayed thoroughly with disinfectant between birds and after visiting a colony. Remove protective clothing, gloves and footwear immediately after exiting a colony and either clean and disinfect them on-site or place them in a plastic bag, or bucket with a lid. Buckets allow soaking in disinfectant before washing. When washing these clothes, rinse first then wash as hot as possible, ideally at more than 60°C, and add a disinfectant such as F10SC.

Rubber boots are advised as they can be easily changed and properly cleaned - ensure that the treads are also cleaned and disinfected, and that no guano has gone inside a boot.

<u>Category B PPE</u>

Same as category A plus:

- Fluid resistant face mask
- Eye protection advised

<u>Category C PPE (for highest HPAI risk)</u>

- Disposable, fluid-resistant suit equivalent to European standard EN 14126, CE certified as Category III, Type 5-B or 6-B (EN 13982-1:2004 or EN 13034:2005) E.g. Tyvek 500 suit
- EN149 **FFP2** or NIOSH **N95** mask
- Goggles that seal against the face
- Non- porous boots, to be cleaned and disinfected when leaving sites / areas of high wildlife density, with appropriate disinfectant.
- Double layered disposable latex gloves used between individuals and outer glove changed each time.

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Any disposable clothing must be soaked in disinfectant before it is disposed of unless it is disposed of directly into a biohazard bin that will be incinerated.

PPE must be donned and removed correctly for it to be effective. Care must be taken not to spread contaminants from the outside of protective clothing when removing it and not to touch the face with contaminated gloves or hands.

The Centre for Disease Control (CDC) in the USA advises on how to "Safely remove PPE in sequence":

1. Remove and dispose of the apron, if worn;
2. Clean and disinfect boots;
3. Remove boots;
4. Remove and dispose of the coverall;
5. Remove and dispose of gloves;
6. Wash hands with soap and water;
7. Remove goggles and respirator;
8. Clean and disinfect reusable goggles and respirator;
9. Wash hands with soap and water again

11.2 Basic Principles of Disinfection

Important factors that affect the efficiency of disinfection are:

1) The target pathogen.
2) Concentration of disinfectant used.
3) Contact time (the pathogen must be exposed to the disinfectant for a minimum period before it is killed).
4) The presence of organic material or other dirt.
5) Material to be disinfected (more porous or rough materials may provide "hiding places").

Few disinfectants work optimally in the presence of organic material, so faeces and dirt should be rinsed, wiped, or scraped off as much as possible before the disinfectant is applied. Where possible, clean equipment with soap and water before applying disinfectant.

The disinfectant must be mixed to the correct concentration required to kill the target pathogen and enough contact time must be allowed. Guidance should be provided on

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product packaging. If it is not possible to clean all the dirt off first, increase the concentration and/or contact time as much as possible. If a piece of equipment is disinfected after touching an infected bird but is used on another bird before the contact time has elapsed, there is a risk that the second bird may be infected. For some disinfectants, the contact time can be reduced by increasing the concentration, but all disinfectants differ in the extent to which this is possible. For some, the concentration must be increased drastically to make any significant reduction in contact time.

Footbaths are a possible means of disinfecting footwear. However, maintaining the correct concentration and cleanliness is difficult and sufficient contact time may be impossible. Mechanical cleaning and removal of dirt may be more effective, and changing footwear may be necessary. Contaminated footwear can then be soaked in disinfectant for sufficient time.

Please dispose of unused disinfectant in an environmentally responsible manner. Please make every attempt to avoid dripping disinfectant onto the ground and do not discard any disinfectant in the field. All disinfectant should be disposed of into a municipal sewerage system.

11.3 Recommended disinfectants for HPAI virus

Virkon S

- use for equipment/ vehicles
- Mix 20g powder in 1L water (2% solution).
- Contact time 7-10 minutes.
- May damage metal so do not leave it on for more than half an hour or use plastic equipment where possible.
- May be an irritant to the skin.
- Available as 50g sachets.
- Can be purchased from farmers' co-ops.

F10 SC

- Less irritant to skin, so can use to disinfect hands and arms.
- Mix **2ml in 1L of water** (1:500 dilution): **10 minutes contact time** (this is described on the label as the dilution for viruses (e.g., Newcastle disease)).

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- Mix **4ml in 1L of water** (double concentration = 1:250 dilution) for **5 minutes** contact time (but this will double your costs and F10 can be expensive).
- F10 hand gel and soap are also available. Also causes some rusting of metal equipment but the F10 Sterilant with rust inhibitor is another option, though more expensive.

Purchase from Veterinary Suppliers:

- VetServ (021 557 2955).
- Lion Bridge (www.lionbridge.co.za).
- Lakato (021 944 6900).
- Health and Hygiene (011 474 1668, info@healthandhygiene.co.za, www.healthandhygiene.co.za).

Household bleach (Sodium hypochlorite)

- Mix 100ml of bleach in 1L of water (= “10% bleach”. Bleach is available at 3.5% concentration in South Africa (e.g. Jik) so this creates a 0.35% solution of sodium hypochlorite).
- Contact time 12-15 minutes.
- In the presence of a lot of dirt and organic material, the concentration needs to be increased to 2-3% and the contact time should be 10-30 minutes. To mix 3.5% bleach to a 2% solution, mix 600ml bleach with 400ml water.
- Corrodes metal (rinse off after half an hour), may discolour the fabric or may irritate the skin.
- For working in in seabird colonies, use products without added detergents or other ingredients.

11.4 Suggested Disinfecting Equipment

- A bucket with a lid to transport and soak equipment and clothing in disinfectant solution.
- A 500ml spray bottle or knapsack sprayer if larger volumes of disinfectant are required, for example for vehicles.
- Scrubbing brushes for equipment and footwear.
- A cloth soaked in disinfectant in a waterproof container for applying disinfectant (keep as wet as possible).

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12. MANAGEMENT OF SEABIRD COLONIES AT VARYING RISK OF HPAI

The table below shows some expected possible scenarios, with varying levels of HPAI outbreak in a seabird colony, extending to possible marine mammal infections. Extra actions may be required as the situation worsens, and other activities may need to be restricted, to protect animals and humans.

*Adapted from the Government of South Georgia & the South Sandwich Islands' **Biosecurity Handbook,** revised October 2023, with thanks.*

	Level 0 **No HPAI**	**Level 1** **HPAI in SA, few seabirds**	**Level 2** **HPAI in seabirds, with increased mortality**	**Level 3** **Mass mortality in seabirds**	**Level 4** **Mass mortality in mammals**
Regional spread of HPAI	No reported HPAI in South Africa in any species the previous 2 months	HPAI confirmed in South Africa in any species in the previous 2 months	HPAI confirmed in seabirds		
Indications / mortality (in the colony in question, unless otherwise indicated)	Mortality not above baseline No individuals showing HPAI signs	Mortality not above baseline 1 or 2 individuals showing possible HPAI signs	Above baseline mortality is suspected but not considered mass mortality Numerous individuals are displaying HPAI signs	Wide-spread mortality across different life stages and/or species. Multiple HPAI signs present in significant number of individuals	Mass mortality in both birds and mammals across multiple sites, confirmed due to HPAI
Media/ public communication	None	None	Consider requesting the public to report suspected cases and take precautions with affected birds	Notify the public of the situation and response in place and request assistance and caution	Notify the public of the situation and response in place. Inform about the raised risk of human infection and advise no contact with affected birds or mammals.
Visitors/ tourists	All sites open as normal.	Site(s) remains open	Site(s) remain open. Consider warning/ information signs	Affected site(s) closed to visitors	All sites closed

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Annex 4.2: Seabird HPAI Protocol

	Level 0 **No HPAI**	**Level 1** **HPAI in SA, few seabirds**	**Level 2** **HPAI in seabirds, with increased mortality**	**Level 3** **Mass mortality in seabirds**	**Level 4** **Mass mortality in mammals**
All activities within colony but more than 5m from animals	Operations as normal	Operations as normal with added vigilance	Projects may continue under caution with category A PPE	Activities reviewed and high priority projects may continue under caution with category B PPE	Activities reviewed and high priority projects may continue under caution with category B PPE (?)
All activities within colony and less than 5m from animals	Operations as normal.	Permits to continue with category A PPE	Projects may continue under caution with category A PPE	Activities reviewed and high priority projects may continue under caution with category B PPE	Permits for animal approach in infected species/colonies to stop Permits reviewed and with DFFE agreement in exceptional circumstances monitoring may continue under caution with Category C PPE
Activities – handling healthy animals	Permits to continue with category A PPE	Permits to continue with category A PPE	Projects may continue under caution with Category B PPE	Permits for animal handling in infected species/colonies to stop Permits reviewed and with agreement from DFFE, in exceptional circumstances, may continue with Category C PPE in species/colonies with no HPAI	No animal handling
HPAI Monitoring requested for anyone entering the colony	5-minute survey period for HPAI signs prior to starting work in area of high wildlife density. Report suspect cases immediately to colony manager	10-minute survey period for HPAI signs prior to starting work in area of high wildlife density Report suspect cases immediately to colony manager	Trained staff in category A PPE should monitor ongoing mortality and symptomatic individuals at site, at least weekly but preferably daily.	Trained staff in category B PPE should note ongoing mortality and symptomatic individuals at site at least weekly but preferably daily. Observers should remain >5m from wildlife.	Trained staff in category B PPE should note ongoing mortality and symptomatic individuals at site at least weekly but preferably daily. Observers should remain >5m from wildlife.

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Annex 4.3

PROTOCOL FOR THE MANAGEMENT OF HIGH PATHOGENICITY AVIAN INFLUENZA (HPAI) FOR MARION ISLAND

Updated: April 2024

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1. PURPOSE

The purpose of this protocol is:

a) To aid early detection of high pathogenicity avian influenza (HPAI) outbreaks and to assist with quantifying their effects.
b) To provide guidance for the management and handling of wildlife susceptible to and infected with HPAI.
c) To implement biosecurity measures to mitigate the spread of and mortality caused by the virus.
d) To reduce the risk to human health.

2. BACKGROUND

High pathogenicity avian influenza (HPAI) has in recent years caused unprecedented mortality of wild birds worldwide, including in South Africa, with threats to population levels for some species already under multiple anthropogenic pressures. HPAI is a disease caused by a virus and is highly contagious in birds. It can be spread over long distances, across and between continents, by wild water birds.

Since 2017, HPAI has caused millions of deaths in commercial chickens and severe damage to the ostrich industry in South Africa. To date, over 30,000 South African seabirds have been affected, mainly Cape Cormorants, Swift Terns, and African Penguins. Thousands of deaths have been recorded in seabirds and marine mammals (mainly South American Sea Lions and Southern Elephant Seals) in South America since December 2022. In October 2023, HPAI (H5N1) was detected in seabirds and marine mammals on the island of South Georgia and in Antarctica in February 2024, in skuas. By the end of January 2024, at least 200 Gentoo Penguins had died from HPAI in the Falkland Islands and the virus was also detected in Gentoo and King Penguins on South Georgia. At least 50 Wandering Albatrosses also died on South Georgia early in 2024 and other procellariiform species affected in the region include Southern Fulmars and Black-browed Albatrosses in the Falklands. Virus was detected in Antarctic Fur Seals and Elephant Seals on South Georgia. From the Antarctic Peninsula, there have also been suspected cases in Antarctic Fur Seals, Gentoo Penguins, and Kelp Gulls. Recent reports can be viewed on the website of the Sub-Antarctic and Antarctic highly pathogenic avian influenza H5N1 monitoring project.

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The risk to seabirds and marine mammals breeding at the Prince Edward Islands (comprising Marion and Prince Edward) has therefore also increased.

This protocol is recommended in the Prince Edward islands Management Plan, and some aspects are covered by Section 2.2.15 “Wildlife disease management” and Section 5.5 “Prevention and control of outbreaks of disease among animals.”

3. HPAI VIRUS TRANSMISSION AND SPREAD

Avian influenza (AI) viruses are present in guano and discharges from the mouth, nostrils, and eyes of infected birds. Any bird carrying the HPAI virus can spread the disease to other birds through direct contact, or respiratory droplets, when in they are close together. The virus is therefore able to spread rapidly between birds that spend extended periods close together, such as in dense seabird colonies. Infected animals will also have the virus present in tissues and organs and can be a source of virus for other birds or mammals that consume them (predators or scavengers). Mammal to mammal transmission is suspected in seals but has not yet been proven.

The virus can also be spread through water contaminated by guano/faeces and possibly carcasses or via contaminated objects, including equipment, vehicles or humans with contaminated clothing or hands. The virus survives particularly well (up to months) in cool, dark conditions and in damp areas and organic matter such as guano or mud. High salinity conditions and extremes in pH shorten virus survival time. The virus is therefore inactivated quicker in salt water but can survive extended periods in fresh water, especially at low temperatures. Contaminated standing fresh water may act as a source of the virus.

4. HPAI IN PEOPLE

Between January 2021 and December 2023, worldwide there were at least seven human infections with H5N1 HPAI of the Clade 2.3.4.4b strain; five infections were severe. The risk to humans is therefore still low but has increased from previous strains, and a much higher number of other mammals has been infected. Mammal-to-mammal transmission has not been proven but is a possibility, with the large numbers of marine mammals affected in South America.

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People slaughtering poultry and those in close contact with infected birds are at highest risk. Infection could occur through contact with body fluids that enter the eyes, nose or mouth as aerosol or droplets, especially if carcasses are opened. People need to take precautions to not only prevent spreading the virus to other birds but also to protect themselves (see Section 12). Strict health and safety measures should be employed for those handling infected birds and materials. It is also advised that seasonal flu vaccinations are received, to reduce the chance of avian and human flu viruses combining.

Although the risk for human infection with HPAI is considered low, people who develop flu-like symptoms within 10 days of being in contact with potentially infected animals should seek medical attention. They should inform a healthcare professional that they have possibly had exposure to avian influenza virus. The medic can consult the National Institute for Communicable Diseases (NICD) on the clinician Hotline number (0800 212 552).

Advice from the NICD on HPAI in humans is available on their website, as well as general advice on influenza, which includes early treatment with oseltamivir (Tamiflu).

Nasal swab samples should be taken from symptomatic people who are suspected to have been exposed to HPAI. Swabs with instructions will be provided by the NICD. After sampling, swabs should be inserted into tubes containing DNA/RNA Shield or RNA*later* (the same tubes that will be used for animal samples), which must be clearly labelled with the patient's name and the date. The swab handles need to be cut to fit into the tubes. Ensure the scissors/ side cutters used to cut the handle are washed, soaked in bleach solution (see section 12.3) for 15 minutes and allowed to dry before use (do not cross-contaminate from animal samples). An NICD sample submission form must be completed with the patient's details and can be inserted into a zip-lock bag and into the bag with the sample tube and frozen at -20°C. A photograph of the completed form and labelled tube should also be kept as back-up.

5. CLINICAL SIGNS OF HPAI

Infections with HPAI in **seabirds** have mainly presented as neurological signs due to brain damage, varying from depression and unusual docility or tameness, although

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subtle twitching and tremors to obvious abnormalities such as walking or swimming in circles, whole-body tremors and seizures or fits occur in severe cases.

Some signs of respiratory infections, including nasal and ocular discharge and swollen sinuses have been seen and behaviour such as open-mouth breathing, nasal snicking (coughing), sneezing, gurgling or rattling may be observed.

Some birds have eyes that appear cloudy, due to corneal oedema and recovered Northern Gannets in Europe were observed to have dark irises.

A video of clinical signs in seabirds can be found at this link:
https://photos.app.goo.gl/bHh7oH4HLbpFrcBZ7

Marine mammals affected by HPAI show neurological signs including difficulty moving, muscle tremors and spasms, uncoordinated movements, seizures (fits) and lack of response to stimuli and their surroundings. Respiratory signs include difficulty breathing, including abdominal breathing, mucus in the mouth or excessive salivation, discharge from the nose, coughing and sneezing. There is also an association with abortions and diarrhoea.

Please take videos of any suspicious behaviour or clinical signs and share them with people who have experience with HPAI (see Annex 4.3.1 for contacts) to decide whether HPAI is likely and whether sampling is justified.

6. OUTBREAK DETECTION AND MONITORING

Surveillance is essential to detect and respond to HPAI outbreaks as early as possible and to track the severity of outbreaks when they occur. This will allow the appropriate response measures to be put in place and the effects of the virus to be quantified and distinguished from other pressures on marine top predator populations. A good knowledge of normal, baseline mortality rates can assist with early detection of an HPAI outbreak, as an increase in mortality will be easily detected through regular monitoring. Effective monitoring relies on trained and alert people, who recognise and report signs of HPAI. Regular patrols should be undertaken at sites where animals

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gather for breeding, roosting, bathing, etc. Please review the work plan for the year and ensure that most sites will be seen regularly.

Laboratory testing is needed to confirm that the HPAI virus is present in a population, although there can be a strong suspicion based on observation of typical clinical signs and a drastic increase in mortality rate. Genetic study of viruses detected can also assist with understanding the possible origins and routes of virus transmission. Once an outbreak has been declared, based on laboratory testing or clinical signs and mortality rate, dead animals which are found with no other clear cause of death may be reported as suspected HPAI cases.

7. REPORTING

If any sign of HPAI is observed (unusual numbers of carcasses without an obvious cause of death or animals behaving strangely), first don appropriate PPE (at least mask and goggles) before further investigation or touching any suspect animal.

For any dead animal or animal that appears ill, the following must be recorded:

a) Date observed
b) Exact location (GPS coordinates in decimal degrees)
c) Species
d) Number of animals affected per species
e) estimated percentage of total colony affected (dead and ill) OR current estimated colony size (individuals) (if possible/ applicable)
f) Estimated size of area affected (if possible/ applicable)
g) Age category (e.g. chick/ juvenile/adult)
h) Whether the animals were found alive or dead
i) If alive, what clinical signs were they showing?
j) If dead, what state of decay were they found in?
k) What was done with the animal, if anything?
l) Name and contact details of the person who made the observation, if further information is required
m) Photographs of dead animals should be taken to assist with species and age identification and determining state of decomposition. If the correct PPE is available, photograph birds from above and below, with the wings stretched out

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and the tail spread, as well as a side view of the head. Include any clinical signs (e.g. nasal discharge, swollen head) and include a sample label in a photo, if samples were taken.

If possible, reports of dead animals or animals showing HPAI symptoms should be made directly on the Department of Forestry, Fisheries and the Environment's (DFFE) Seabird Avian Influenza Reporting Tool:

- The Survey123 App can be downloaded from the Google Play Store or the Apple App Store and then the project can be loaded using the QR code below.

- Alternatively, you can follow this link in a web browser: **https://arcg.is/1585011** and open the App to link to the project.
- **Please select "Continue without signing in," on the Survey123 login page.**
- The reported data can be viewed on a dashboard, web map, and database here. Data can be downloaded as a CSV file (under Options) from the web map page.

If reporting in this way is difficult, a reporting template is available as Annex 4.3.2 and can be completed in Microsoft Excel (which can be shared online) or printed and completed on paper.

In terms of the PEI Management Plan (p146), suspected disease outbreaks (increased numbers of animals with no obvious cause of death or animals showing clinical signs) must immediately be reported to the Environmental Control Officer (ECO) and Team Leader (TL), who appoints a Field Coordinator for further monitoring and response. Regular reports should be made to an HPAI steering committee, appointed to advise remotely and final collated information should be supplied in the annual ECO report. A Field Coordinator and outbreak monitor/record-keeper should be appointed at takeover, at the start of the overwintering period. These can be the same person but

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all reports from the island should be collated and samples should be organised by the same person.
The HPAI steering committee should comprise at least one representative from the PEI Management Committee, the principal investigators, a SAPRI representative, the field coordinator and experts with experience with avian influenza in marine animals.

Confirmed or suspected cases of HPAI should be reported internationally to the World Organisation for Animal Health, via Western Cape provincial Veterinary Services and the national Director: Animal Health, Department of Agriculture, Law Reform and Rural Development's (DALRRD). Though it may not be possible to confirm HPAI with laboratory testing until samples return to South Africa, suspected cases should be reported. The PE Islands are technically part of Ward 115, City of Cape Town and therefore fall under the office of State Veterinarian: Boland. Suspected HPAI outbreaks and/or cases can be reported to either the responsible state vet or a State Vet: Epidemiology. Contact details are available on the Directorate Animal Health, DALRRD website and in Annex 4.3.1. Additionally, information should be provided to the SCAR Antarctic Wildlife Health Network via the SA SCAR representative.

It is important that no one discusses suspected cases with anyone outside the overwintering team and the HPAI steering committee, until an official public announcement has been made.

After the outbreak is considered over, the overwintering team must carry out post-event monitoring as instructed by D: SO&AS.

8. SPECIMEN COLLECTION FOR LABORATORY TESTING

Detailed instructions for sampling are attached as Annex 4.3.3 and equipment required in Annex 4.3.4.

1.1. Introduction

Confirmation of HPAI infection is done through specific laboratory tests, most commonly a PCR test to detect the RNA (genetic material) of the HPAI virus. Testing for avian influenza virus in South Africa can only be done in laboratories that are approved by the national Directorate Animal Health of DALRRD. Surveillance done for

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research purposes also requires approval from this directorate, under Section 20 of the Animal Diseases Act No. 35 of 1984.

1.2. Sampling live birds

To confirm if live birds, that are showing clinical signs of HPAI, are affected by the HPAI virus, oral/ pharyngeal and/or tracheal and cloacal swabs can be collected. If the bird cannot be handled for sampling, fresh guano can be sampled after the bird has moved away, if a sample can be linked with certainty to a specific bird. Insert the swab into the faecal component and avoid the white urate portion.

To date, nasal and rectal swabs from live marine mammals have been a poor source of virus in infected individuals, so sampling live mammals is not described.

Where possible, take a video and consult with an experienced person before sampling. Otherwise try to make sure that the bird is showing consistently abnormal behaviour, or that there is more than one bird behaving strangely, before sampling.

1.3. Sampling dead animals

Brain swabs should be collected from dead animals suspected to have died from HPAI. Sampling of the lung and lower trachea is important in marine mammals and if neurological clinical signs are not dominant but HPAI is still suspected. Techniques to take these samples as safely as possible, without opening the whole carcass, are described in Annex 4.3.3.

Samples should be taken within 3 days of when the animal died, but brain samples may still be useful after a longer period, if environmental temperatures have been low and sampling sooner is impossible.

If an abnormally large number of carcasses is noted, without a visible cause of death, samples can be taken from up to 3 animals. Try to sample animals from across the affected area.

1.4. Sample transport

All samples should be kept cool but not frozen and protected from direct sunlight. Where immediate (within 7 days) transport to a laboratory is not possible (e.g. due to

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long voyage times), specialised transport media like DNA/RNA Shield or RNA*later* must be used to allow for long-term storage by freezing samples at -20°C.

1.5. Sample records

Please keep careful records of samples taken so that test results can be matched to the information about the sampled animal. Photograph field notebooks as a backup and enter the information in a sample list (Excel template supplied in Annex 4.3.2). The information can also be added in the App.

1.6. Sample numbers

There will be limited consumables available for sampling so they should be used carefully. **Three live and three dead individuals of each species** will be enough, once the mortality rate and clinical signs have been discussed and concluded to be consistent with HPAI.

9. CARCASS REMOVAL

The carcasses of animals that have died from HPAI are potential sources of the HPAI virus which can infect other animals, especially scavengers. Removing these carcasses may reduce the quantity of the virus in the environment and reduce the risk of infection in other animals. Moving carcasses away from targeted areas such as freshwater bathing and/or drinking sites or high-traffic areas (e.g. pathways) may be especially effective. However, the contribution of carcasses to the overall amount of the virus in the environment may be of far less importance that the amount shed by infected animals and the feasibility and harmful effects of carcass collection and disposal should be weighed against the possible benefits.

Carcass removal should only be done if all the following criteria can be met:

1) A safe and effective carcass disposal option is available (e.g. burial: see section 10).
2) Carcasses can be collected without excessive disturbance of healthy animals. Such disturbance may increase the transmission of the disease if it leads to changes in behaviour (especially if animals crowd together or disperse to other healthy colonies) or stress. Disturbance can cause severe negative effects which are unrelated to the disease.

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3) Sufficient personnel are available to collect carcasses.
4) Carcass removal can be done with minimal risk to people. Risks can be related to dangerous terrain or a virus with high potential to transmit to humans. Anyone handling potentially infected carcasses should wear suitable protective clothing (see section 12.1 for details).
5) Carcass removal and disposal can be done without increased risk of spreading the disease to new areas or species.
6) Fresh carcasses can be collected or frequent collection can be organised, as carcasses will be most infectious soon after the bird dies.

Accurate records must be kept of the number of carcasses collected, the date, time and location collected, species, ages, and transport and disposal methods (see section 7).

10. CARCASS DISPOSAL

Carcass disposal options are extremely limited, which will limit the number of carcasses that should be removed. The only recommended action to consider is therefore moving some carcasses to an area where there is a lower probability that they will cause harm. If possible, first discuss with the HPAI steering committee, so the risk can be assessed.

Carcass disposal options include:

A. Burial: sufficient space and suitable substrate is required to ensure that a hole can be excavated and the carcasses will not cause contamination of soil or water sources. There are few suitable sites on Marion Island, and may impact ecological functioning, so this is not recommended.
B. Composting - sufficient plant material is required to achieve the correct carbon-nitrogen ratio and the correct moisture level. This is not available on Marion Island.
C. Incineration/burning is not allowed on Marion Island without permission.
    a. Incineration in an incinerator: the machinery and fuel must be available and the processing capacity of incinerators can be limited. This is unlikely to be a suitable option for mass mortality events.

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b. Burning: sufficient fuel and airflow (possibly burning on a platform) is required to maintain sufficient heat.

11. SICK ANIMALS

Sick animals should not be handled or moved except for sampling. Their removal is unlikely to cause a significant reduction the amount of virus in the environment, as not all sick animals will be observed and they will begin shedding virus before they appear ill.

Treatment is also unlikely to be feasible and will be ineffective in many cases. Euthanasia is not common practice on Marion Island and is not recommended. No methods of euthanasia is painless, and training and practice is required to ensure that the animal does not suffer unnecessarily.

Any handling of sick animals poses a risk to human health and to healthy animals that may be handled afterwards.

12. BIOSECURITY GUIDELINES

Anything that touches an infected bird or contaminated object, or environment may transfer the HPAI virus to a healthy bird. Anything that is visibly dirty poses the greatest risk, so every attempt should be made to ensure that footwear, clothing and equipment are cleaned of visible dirt as soon as possible after leaving an area densely populated by animals, and that, wherever possible, disinfectant is applied afterwards. Try to keep equipment and clothing as clean as possible e.g. minimise the number of objects that touch the ground and do not sit on the ground in the colony. Please do not discard any unused or excess disinfectant in the field and under no circumstances allow it to enter streams or dams.

HPAI virus is present in seabird colonies in South Africa but is currently not often visible as clinical cases. Therefore, new equipment that has not been in contact with South African birds should be used and older equipment should be thoroughly cleaned and soaked in disinfectant before use.

Vessels and aircraft should also be cleaned and disinfected before moving from one part of a colony to another and between colonies, especially the foot wells, wheels,

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and undercarriage. Dirty equipment and clothing should be cleaned before transport, or packaged to prevent contamination of rucksacks, or the vessel or aircraft.

12.1 Clothing and personal protective equipment (PPE)

Biosecurity and PPE recommendations below have been matched to HPAI risk level for different activities and disease situations. They are designed both to protect staff and prevent virus spread between sites and species. Individuals may choose to adopt a more precautionary approach, but this is considered the minimum. PPE categories increase as the probability of HPAI virus exposure increases (highest for handling infected animals) and the risk of human infection rises (highest after there have been detections in other mammal species).

When washing potentially contaminated clothes, rinse off as much visible dirt as possible, soak in disinfectant for at least half an hour, and wash as hot as possible, ideally at more than 60°C, or add a disinfectant such as F10SC.

Rubber (gum) boots are advised as they can be easily changed and properly cleaned. Ensure that the treads are also cleaned and disinfected, and that no guano/dirt has gone inside a boot. At all times, when moving between colonies, clean off dirt and rinse boots wherever possible and ideally also spray with disinfectant (carry a 500 ml spray bottle). If using fresh water, do not wash into the water as you could spread virus. Ideally wash in the sea.

Goggles will be re-used so should be sprayed with disinfectant immediately after use so they can be safely handled, then cleaned and sprayed again. Leave them wet to allow for contact time.

The PPE required is listed in Annex 4.3.4.

<u>Category A PPE (for lowest HPAI risk/ normal activities)</u>

- Overalls/ rain suit/ oilskin jacket and trousers that keep clothes and skin clean and dry underneath and can be easily cleaned and sprayed with disinfectant.
- Wear disposable gloves or clean and sanitize hands/ disinfect gloves between handling individual live animals. Alcohol sanitizers can be used on hands or spray hands/ gloves with F10SC.

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- Footwear and outerwear to be cleaned and disinfected between sites/areas of high wildlife density with appropriate disinfectant. At a minimum, trying to rinse off mud and guano from a colony from footwear.

Clothing should be dedicated to colony work and should ideally be waterproof but should be as impermeable as possible and should have minimal folds and seams and no Velcro that will catch dirt and guano. The PPE should be able to be sprayed thoroughly with disinfectant after visiting a colony.

Category B PPE (for working near infected colonies but without direct contact with animals)

Same as category A, plus:

- Fluid resistant face mask/ respirator
- Eye protection

Category C PPE (for highest HPAI risk- contact with potentially infected animals)

- Disposable, fluid-resistant suit equivalent to European standard EN 14126, CE certified as Category III, Type 5-B or 6-B (EN 13982-1:2004 or EN 13034:2005) e.g. Tyvek 500 suit
- EN149 **FFP2** or NIOSH **N95** respirator
- Goggles that seal against the face
- Non-porous boots, to be cleaned and disinfected when leaving infected sites/areas of high wildlife density, with appropriate disinfectant.
- Double layered disposable latex gloves. Outer gloves changed between handling individual live animals.

Disposable clothing must be sealed in a plastic bag immediately after use, taking care not to contaminate the outside of the bag, and placed into a biohazard bin to be incinerated. PPE must be donned and removed correctly for it to be effective. Care must be taken not to spread contaminants from the outside of the protective clothing when removing it and not to touch the face with contaminated gloves or hands.

The Centre for Disease Control (CDC) in the USA advises on how to “Safely remove PPE in sequence”:

1. Remove and dispose of the apron, if worn;

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2. Clean and disinfect boots;
3. Remove boots;
4. Remove and dispose of the coverall;
5. Remove and dispose of gloves;
6. Wash hands with soap and water;
7. Remove goggles and respirator;
8. Clean and disinfect reusable goggles and respirator; and
9. Wash hands with soap and water again

12.2 Basic Principles of Disinfection

Note: disinfection applies only to clothing, footwear, equipment, vessels and aircraft (man-made objects) and will not be done on any animal or in any colony. No disinfectant is to be discarded onto the ground or into any water.

Important factors that affect the efficiency of disinfection are:

1. The target pathogen.
2. Concentration of disinfectant used.
3. Contact time (the pathogen must be exposed to the disinfectant for a minimum period before it is killed).
4. The presence of organic material or other dirt.
5. Material to be disinfected (more porous or rough materials may provide “hiding places”).

Few disinfectants work optimally in the presence of organic material, so faeces and dirt should be rinsed, wiped, or scraped off as much as possible before the disinfectant is applied. Where possible, clean equipment with soap and water before applying disinfectant. If proper cleaning and disinfection is not possible when leaving the colony, spray with disinfectant and then place in a sealed plastic bag or container, for safe transport and cleaning at a later stage.

The disinfectant must be mixed to the correct concentration required to kill the target pathogen and enough contact time must be allowed. Guidance is provided in section 12.3 and on the product packaging. If it is not possible to clean all the dirt off first, increase the concentration and/or contact time as much as possible. If a piece of

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equipment is disinfected after touching an infected bird but is used on another bird before the contact time has elapsed, there is a risk that the second bird may be infected. For some disinfectants, the contact time can be reduced by increasing the concentration, but all disinfectants differ in the extent to which this is possible. For some, the concentration must be increased drastically to make any significant reduction in contact time.

Please make every attempt to avoid dripping disinfectant onto the ground and do not discard any disinfectant in the field. All disinfectant should be disposed of in dedicated bins for return to South Africa.

12.3 Recommended disinfectants for HPAI virus

Virkon S - Use for hard, nonporous surfaces e.g. equipment/aircraft

- Mix 20g powder in 1L water (2% solution).
- Contact time 7-10 minutes.
- May damage metal so do not leave it on for more than half an hour or use plastic equipment where possible.
- May be an irritant to the skin.
- Available as 50g sachets.
- Can be purchased from farmers' co-ops.

F10 SC – Can be used widely, including on skin

- Less irritant to skin, so can be used to disinfect hands and arms. Some people are allergic, however.
- Mix **2ml in 1L of water** (1:500 dilution): **10 minutes contact time** (this is described on the label as the dilution for viruses (e.g., Newcastle disease)).
- Mix **4ml in 1L of water** (double concentration = 1:250 dilution) for **5 minutes** contact time (but this will double your costs and F10 can be expensive).
- F10 hand gel and soap are also available.
- Also causes some rusting of metal equipment but the F10 Sterilant with rust inhibitor is another option, though more expensive.

Purchase from Veterinary Suppliers:

- VetServ (021 557 2955).

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- Lion Bridge (www.lionbridge.co.za).
- Lakato (021 944 6900).
- Health and Hygiene (011 474 1668, info@healthandhygiene.co.za, www.healthandhygiene.co.za).

Household bleach (Sodium hypochlorite) – For hard, nonporous surfaces e.g. equipment or fabrics where colour loss is not a concern

- Mix 100ml of bleach in 1L of water (= “10% bleach”. Bleach is available at 3.5% concentration in South Africa (e.g. Jik) so this creates a 0.35% solution of sodium hypochlorite).
- Contact time 12-15 minutes.
- In the presence of a lot of dirt and organic material, the concentration needs to be increased to 2-3% and the contact time should be 10-30 minutes. To mix 3.5% bleach to a 2% solution, mix 600ml bleach with 400ml water.
- Corrodes metal (rinse off after half an hour), may discolour fabric or may irritate the skin.
- For working in seabird colonies, use products without added detergents or other ingredients.

Examples of other effective products, with active ingredients listed, are available on the US Environmental Protection Agency (EPA) website.

For use in the field, safer and less persistent disinfectants may be required. The EPA also has a list of active ingredients that are considered more environmentally-friendly. Compounds effective against HPAI virus are listed below and may be used for hard, non-porous surfaces:

- Hydrogen peroxide e.g. Oxivir (1 minute contact time)
- Citric acid
- Peracetic/ peroxyacetic acid e.g. Oxiacid

12.4 Suggested Disinfecting Equipment

- A bucket with a lid to transport and soak equipment and clothing in disinfectant. At minimum, plastic bags for clothing or equipment that needs disposal or cleaning.

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- A 500ml spray bottle or knapsack sprayer if larger volumes of disinfectant are required, for example for aircraft.
- Scrubbing brushes for equipment and footwear.
- A cloth in a waterproof container for applying disinfectant.

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13. MANAGEMENT OF SEABIRD COLONIES AT VARYING RISKS OF HPAI

The table below shows some expected possible scenarios, with varying levels of HPAI outbreaks on Marion Island, extending to possible marine mammal infections. Extra actions may be required as the situation worsens, and other activities may need to be restricted, to protect animals and humans.

*Adapted from the Government of South Georgia & the South Sandwich Islands' **Biosecurity Handbook**, revised October 2023, with thanks.*

	Level 0 **No HPAI in linked locations**	**Level 1** **HPAI in linked locations, few seabirds affected**	**Level 2** **HPAI in seabirds, with increased mortality**	**Level 3** **Mass mortality in seabirds**	**Level 4** **Mass mortality in mammals**
Regional spread of HPAI	No reported HPAI on PEIs, South Africa, Crozet, Gough or Amsterdam Islands	HPAI observed in South Africa, Crozet, Gough or Amsterdam Islands	HPAI observed in seabirds on Marion Island	HPAI observed in seabirds on Marion Island	HPAI observed in seabirds **and mammals** on Marion Island
Indications / mortality	Mortality not above baseline No individuals showing HPAI signs	Mortality not above baseline 1 or 2 individuals showing possible HPAI signs	Above baseline mortality is suspected but not considered mass mortality Numerous individuals are displaying HPAI signs	Wide-spread mortality across different life stages and/or species. Multiple HPAI signs present in a significant number of individuals	Mass mortality in both birds and mammals across multiple sites, strongly suspected or confirmed due to HPAI
Media/public communication **(Draft statement Annex 4.3.5)**	None	None	Release a media statement- SCAR and WOAH should be informed so it cannot be kept secret	Notify the public of the situation and the response in place	Notify the public of the situation and the response in place.
All activities within the colony but more than 5m from animals	Operations as normal.	Operations as normal with added vigilance	Projects may continue under caution	Activities reviewed and high-priority projects may continue under caution with category B PPE	Activities reviewed and high-priority projects may continue under caution with category B PPE, if agreed

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Annex 4.3: Marion Island HPAI Protocol

	Level 0 **No HPAI in linked locations**	**Level 1** **HPAI in linked locations, few seabirds affected**	**Level 2** **HPAI in seabirds, with increased mortality**	**Level 3** **Mass mortality in seabirds**	**Level 4** **Mass mortality in mammals**
All activities within the colony and less than 5m from animals	Operations as normal.	Operations as normal with added vigilance	Projects may continue under caution with category B PPE	Activities reviewed and high-priority projects may continue under caution with category B PPE	Animal approach in infected species/colonies to stop. Permits reviewed and with DFFE agreement in exceptional circumstances monitoring may continue under caution with Category C PPE, if agreed
Activities – handling healthy animals	Operations as normal.	Operations to continue with category B PPE	Projects may continue under caution with Category B PPE	Animal handling in infected species/colonies to stop. Permits reviewed and with agreement from DFFE, in exceptional circumstances, may continue with Category C PPE in species/colonies with no HPAI	No animal handling
HPAI Monitoring requested for anyone entering the colony	5-minute survey period for HPAI signs prior to starting work in the area of high wildlife density. Move to category B PPE if any abnormalities are observed Report suspect cases immediately the ECO and TL	10-minute survey period for HPAI signs prior to starting work in area of high wildlife density. Move to category B PPE if any abnormalities are observed Report suspect cases immediately to ECO and TL	Trained staff in category A PPE should monitor ongoing mortality and symptomatic individuals at site, at least weekly but daily if possible.	Trained staff in category B PPE should note ongoing mortality and symptomatic individuals at site at least weekly but preferably daily. Observers should remain >5m from wildlife.	Trained staff in category B PPE should note ongoing mortality and symptomatic individuals at site at least weekly but preferably daily. Observers should remain >5m from wildlife.

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Acknowledgements

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Azwianewi Makhado, Makhudu Masotla, Millicent Makoala, Daisy Kotsedi, Carol Jacobs (all DFFE), Laura Roberts (Animal Health, Directorate: Veterinary Services, Department of Agriculture, Western Cape Government; University of Pretoria), David Roberts (The Southern African Foundation for the Conservation of Coastal Birds), Liezl Pretorius (University of Pretoria, previously Dyer Island Conservation Trust), Maëlle Connan (Nelson Mandela University) and Peter Ryan.

14. LINKS TO OTHER ADVICE ON HPAI IN ANTARCTICA

World Organisation for Animal Health

Practical guide for authorised field responders to HPAI outbreaks in marine mammals
https://www.woah.org/app/uploads/2024/02/practicalguide-forauthorisedfieldresponders-hpaimarinemammals-feb24.pdf

The Council of Managers of National Antarctic Programs (COMNAP)

Highly Pathogenic Avian Influenza (HPAI) Preparedness, Monitoring & Response
https://www.comnap.aq/heightened-risk-of-hpai-in-antarctica
COMNAP Guidance on HPAI Preparedness in Response to the Heightened Risk (version 1/30 August 2023) from the Member's Only Documents area

Scientific Committee on Antarctic Research (SCAR)

Antarctic Wildlife Health Network (Dewar et al)
Sub-Antarctic and Antarctic highly pathogenic avian influenza H5N1 monitoring project
https://scar.org/library-data/avian-flu

The risk of highly pathogenic avian influenza in the Southern Ocean: a practical guide for operators and scientists interacting with wildlife

International Association of Antarctica Tour Operators (IAATO)

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IAATO 2022-23 Biosecurity Protocols Regarding Avian Influenza
https://iaato.org/iaato-2022-23-biosecurity-protocols-regarding-avian-influenza/

US National Science Foundation (US Antarctic Program (USAP))

Updated Guidance on the Highly Pathogenic Avian Influenza - Environmental Update for USAP Personnel
https://www.nsf.gov/news/news_summ.jsp?cntn_id=308279&org=OPP

European Polar Board

Upcoming issues of importance: Avian influenza
https://www.europeanpolarboard.org/communications/upcoming-issues-of-importance/

Antarctic wildlife: Risk of Avian Influenza
https://www.europeanpolarboard.org/news-events/news/article/news/antarctic-wildlife-risk-of-avian-influenza/

Sites with information on cases
https://www.acap.aq/resources/disease-threats/avian-flu
https://scar.org/library-data/avian-flu

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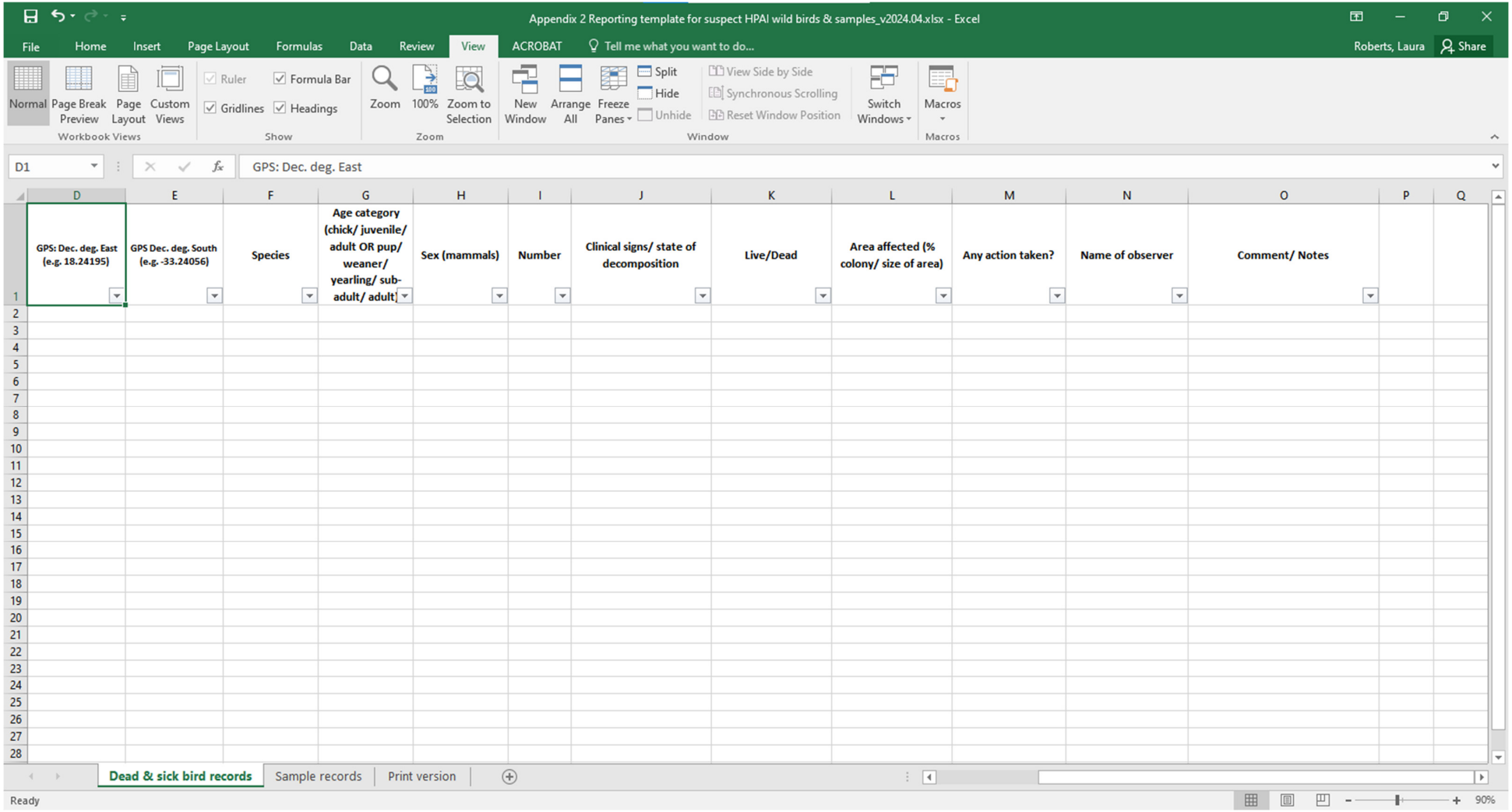
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Marion Island
Role-players and stakeholders

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Western Cape Dept of Agriculture/ UP	Veterinary Services	State Veterinarian: Epidemiology (HPAI management/ monitoring advice)	Laura Roberts, Dr	Laura.Roberts@westerncape.gov.za

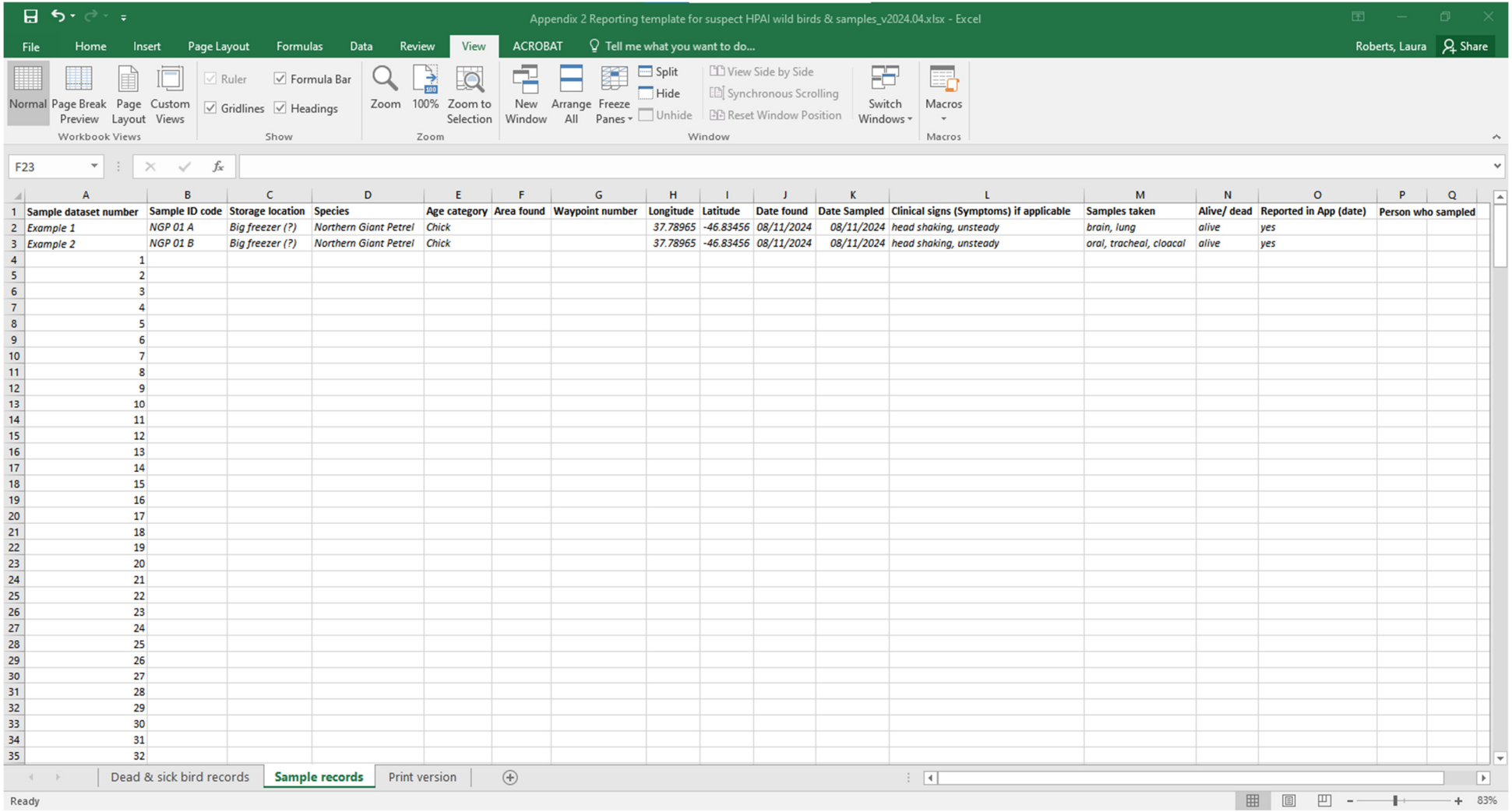
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Annex 4.3.2: Surveillance/ outbreak data collection template



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Annex 4.3.2: Sample records



Appendix 2 Reporting template for suspect HPAI wild birds & samples_v2024.04.xlsx - Excel

Sample dataset number	Sample ID code	Storage location	Species	Age category	Area found	Waypoint number	Longitude	Latitude	Date found	Date Sampled	Clinical signs (Symptoms) if applicable	Samples taken	Alive/ dead	Reported in App (date)	Person who sampled
Example 1	*NGP 01 A*	*Big freezer (?)*	*Northern Giant Petrel*	*Chick*			*37.78965*	*-46.83456*	*08/11/2024*	*08/11/2024*	*head shaking, unsteady*	*brain, lung*	*alive*	*yes*	
Example 2	*NGP 01 B*	*Big freezer (?)*	*Northern Giant Petrel*	*Chick*			*37.78965*	*-46.83456*	*08/11/2024*	*08/11/2024*	*head shaking, unsteady*	*oral, tracheal, cloacal*	*alive*	*yes*	
1															
2															
3															
4															
5															
6															
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32															

Dead & sick bird records | Sample records | Print version

Ready

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Surveillance/ outbreak data
(v. 2024.04)

Name:_____ Page___ of ___

Date (DD/MM/YYYY)	Dec. deg. East (e.g. 18.24195) OR area name/ beach code/ waypoint number	Dec. deg. South (e.g. -33.24056)	Species	Number	Clinical signs	Name & contact details (Email/ Cell) of person reporting	Comments: lab tests / other healthy bird species seen / other relevant information

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When completed please return to your supervisor OR scan/phograph and email to laura.roberts@westerncape.gov.za

UC DAVIS
VETERINARY MEDICINE
Karen C. Drayer Wildlife Health Center

High Pathogenicity Avian Influenza (HPAI) sampling protocol for marine wildlife

Ralph E. T. Vanstreels & Marcela Uhart

January 2024

Personal protective equipment (PPE)

- Full-body coverall with hood (Tyvek or similar)
- Waterproof, washable high-shaft boots (gumboot/Wellington boots or similar)
- Procedure gloves (preferably nitrile); use two layers with the inner layer placed covering and taped to the coverall sleeves and the outer layer being replaced between carcasses or whenever soiled or torn. Ideally use gloves of different colors for each layer to better identify tears or other defects in the outer layer.
- Tape (preferably duct tape); use to seal the inner set of gloves to the coverall sleeves
- Mask (N95, KN95 or PFF-1)
- Protective eyewear (vented goggles or face shield)
- See here for donning and doffing procedures

Sample collection and documentation materials

- Sterile swabs with synthetic fibre (preferably polyester) and breakable synthetic handle (preferably polystyrene)
- Sterile plastic vial with screw cap (preferably cryotube with external thread) containing 1 mL of virus-inactivating storage medium (preferably DNA/RNA Shield® since it inactivates the virus plus has minimum cold chain constraints)
- Sample bags with various sizes (Ziploc®, Whirl-Pak®, etc.)
- Stainless steel necropsy instruments: knife (preferably 6" narrow stiff boning knife), knife sharpener (honing steel or similar), scalpel handle, disposable scalpel blades, scissors, tweezers, etc.
- Small squirt bottle (250 mL) with 70% ethanol; use to soak feathers during sampling procedures
- Digital camera (preferably waterproof so it can be thoroughly washed and disinfected)
- Notebook or white PVC board; use to take notes
- Tracing paper (preferably 180 g or thicker); use with pencil for sample identification
- Alcohol-resistant permanent markers, pencils and pencil sharpener
- Portable cooler with reusable ice packs

Cleaning, disinfection and disposal

- Hand scrub brush
- Liquid detergent
- Water (2–5 liters per person)
- Spray bottle with disinfectant (Virkon-S®, 5% bleach, peroxides, QACs, Lysoform®, Vyrox®, etc.)
- Biohazard infectious material bags (red)

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1. Photographic documentation. The goal is to obtain images that will be useful to confirm the identification of the host species, sex and age class. If possible, include a full-body photograph with a scale or an object that can be used to estimate body size (Fig. 1A). The photographs should show all the anatomical characteristics that are relevant for species/sex/age identification (bill, legs, dorsal and ventral plumage, genitals, etc.; Fig. 1B). If the carcass has characteristics linked to antemortem clinical signs (ocular or nasal secretions, diarrheic feces, atypical body posture, etc.), ensure that these are clearly visible in some of the photographs (Fig. 1C). When sampling individuals of the same/similar species, take a photo that includes the identification next to the animal (Fig. 1D).



Fig. 1. Photographic documentation of sampled specimens.

A – Known-size objects (such as a necropsy knife) can be used as a scale in photos.
B – Anatomical characteristics relevant for species/age/sex determination (such as dorsal plumage in shorebirds) should be carefully photographed.
C – Clinical signs (such as diarrhea-stained peri-cloacal feathers in birds) should be documented when present.
D – To ensure that the photos can later be correctly assigned to each individual, it is useful to take a photo where both the carcass and the sample identification are visible.

2. Swab from the upper respiratory tract. These should be collected before handling the carcass excessively, to avoid contamination with sand/soil. For birds, open the bill and rub the swab on the surface of the larynx; in dead birds, the swab should be superficially introduced (<1 cm) into the opening of the larynx (Fig. 1A). For pinnipeds, the swab should be deeply introduced (5 – 10 cm) into the nostril to reach the inner nasal mucosa (Fig. 1B), and then it is inserted into the lateral mouth and rubbed against the gum (Fig. 1C). For cetaceans, the swab should be introduced into the blowhole as deep as possible (Fig. 1D). In all cases, rotating motion should be used to rub the swab on the surface of the respiratory mucosa, ensuring that secretions are absorbed by the swab tip.

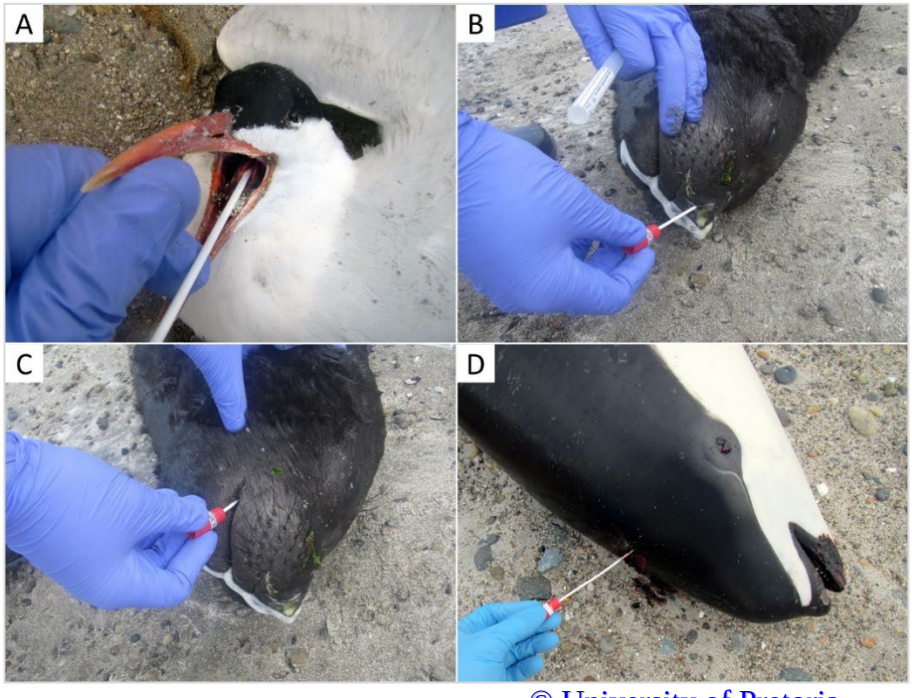


Fig. 2. Collection of swabs from the upper respiratory.

A – Orotracheal swab from a tern.
B – Nasal swab from an elephant seal pup.
C – Oral swab from an elephant seal pup.
D – Blowhole swab from a porpoise.

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3. Swab from the terminal digestive/genital tract. For birds, alcohol may be used to collapse the feathers and make it easier to find the cloaca; then, the swab is superficially introduced (<1 cm) into the cloaca (Fig. 3A). For pinnipeds, the tail is lifted and the swab is inserted directly into the rectum (Fig. 3B); in young pinnipeds, it should be borne in mind that the genital opening and the anus are very closely positioned in the anogenital area, hence the area needs to be closely inspected when inserting the swab. For cetaceans, the swab should be inserted in the anus, which is located caudally to the genital slit (Fig. 3C). Optionally, a swab can also be obtained from the genital slit of cetaceans (Fig. 3D).

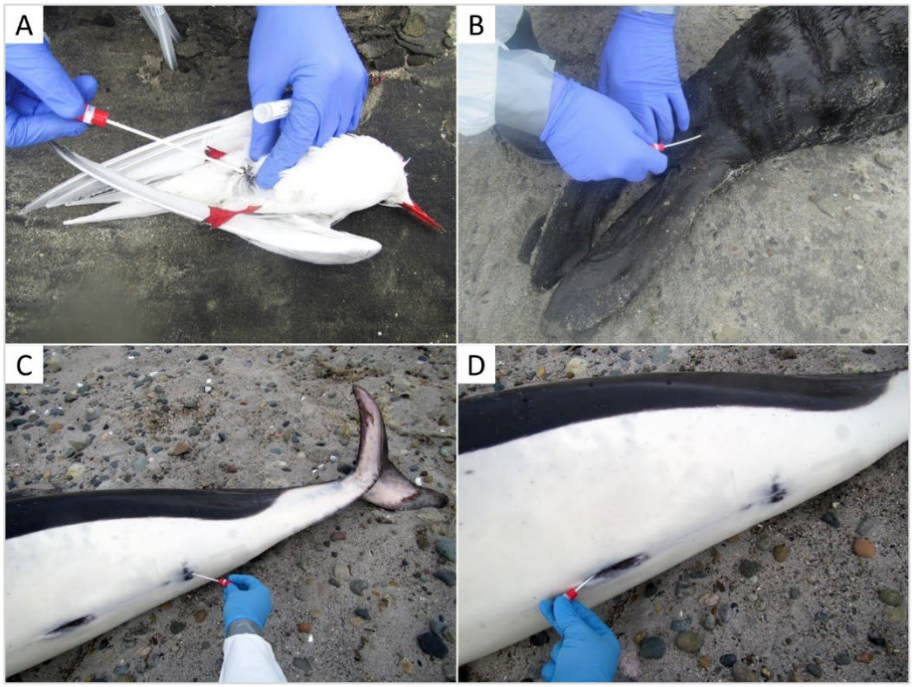


Fig. 3. Collection of swabs from the terminal digestive/genital tract.

A – Cloacal swab from a tern.
B – Rectal swab from an elephant seal pup.
C – Rectal swab from a porpoise.
D – Genital slit swab from a porpoise.

4. Swab from the trachea. Tracheal swabs may be difficult to collect from birds due to their small size and from cetaceans due to their anatomy, however they can be consistently collected from pinnipeds of all sizes. The animal should be positioned in lateral or dorsolateral decubitus. The larynx should be located through palpation, and then a transversal cut should be made to the throat, to open the trachea (Fig. 4A and 4B). Then, a swab is inserted caudally into the trachea, rubbing the tip against the inner surface of the trachea (Fig. 4C); it is often helpful to tilt the head backwards in order to better expose the trachea. Some animals will have considerable amounts of mucus or fluids in the trachea; when possible, these should be photographed (Fig. 4D).

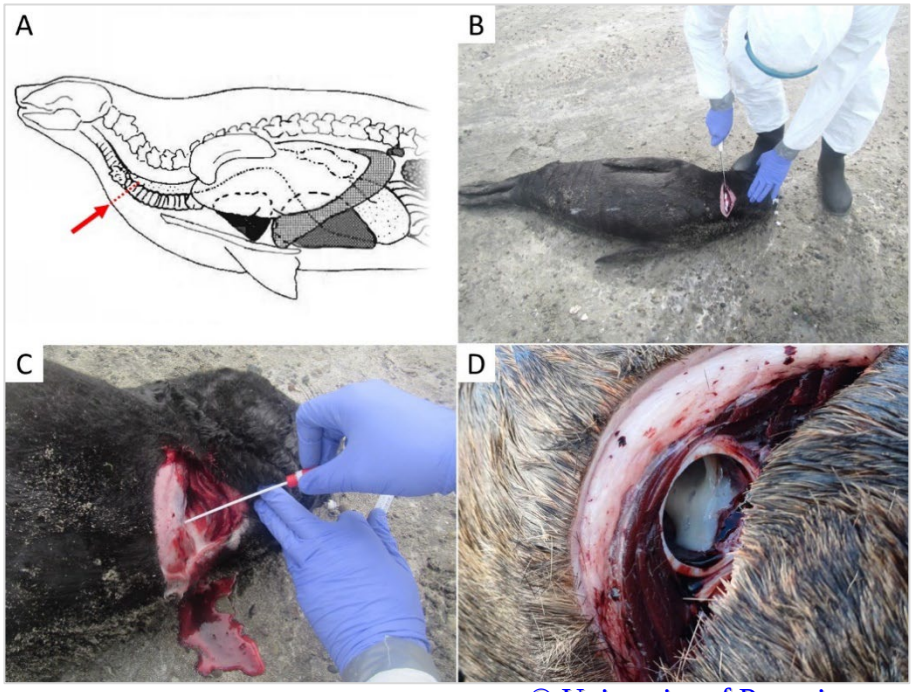


Fig. 4. Collection of swabs from the trachea of pinnipeds.

A – Anatomical site where the throat should be cut in pinnipeds.
B – Cutting the throat of an elephant seal pup, with the carcass in dorsolateral decubitus to facilitate access.
C – Insertion of the swab into the trachea of an elephant seal pup.
D – Photographic documentation of the accumulation of mucus in the trachea of a sea lion.

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5. Swab from the lungs. Lung swabs can be obtained by opening a "window" to the thorax, with the carcass in ventral decubitus. In pinnipeds, the vertebrae and the scapula are used as landmarks, with the "window" starting caudal to the end of the scapula, cutting parallel to the vertebral spine and then ventral before the end of the ribcage (Fig. 5A). In birds, the insertion of the vertebrae and the wings are used as landmarks, with the window starting caudal to the shoulders (Fig. 6A). After cutting a flap of skin in these areas (Fig. 5B and 6B) the thorax is accessed by opening a gap between the ribs. In birds and young pinnipeds, this is made easier because the ribs are not entirely calcified, and therefore the ribs can be cut to create a wider access into the thorax (Fig. 5C and 6C); the dorsal extremity of the ribs should be avoided as it tends to bleed more profusely. In adult pinnipeds, access to the thorax will be limited to the space between the ribs. Once the lung has been exposed, a cut should be made to allow the insertion of the swab into the lung parenchyma (Fig. 5D and 6D).

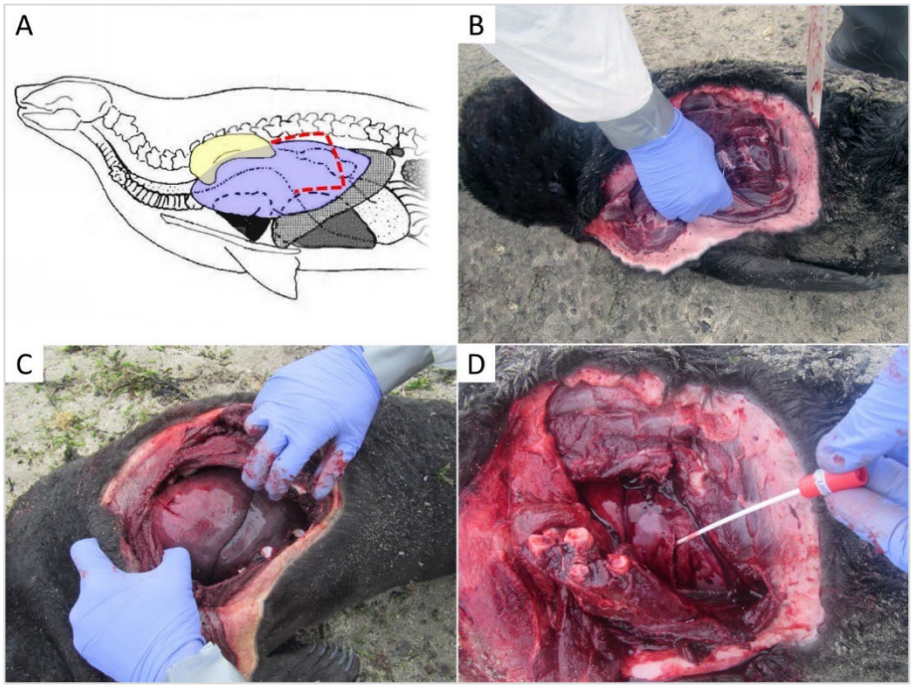


Fig. 5. Collection of swabs from the lungs of pinnipeds.

A – Anatomical site where the "window" (red dashed line) should be opened in the dorsum, starting caudal to the scapula (yellow) in order to obtain a swab from the lungs (blue) of pinnipeds.
B – Cutting a "window" on the torso of an elephant seal pup, with the carcass in ventral decubitus.
C – After the ribcage has been open, the ribs can be forcibly separated (or cut with a knife in pups) to expose the lungs.
D – After a cut is made to the lung, the swab is inserted to sample the lung parenchyma.

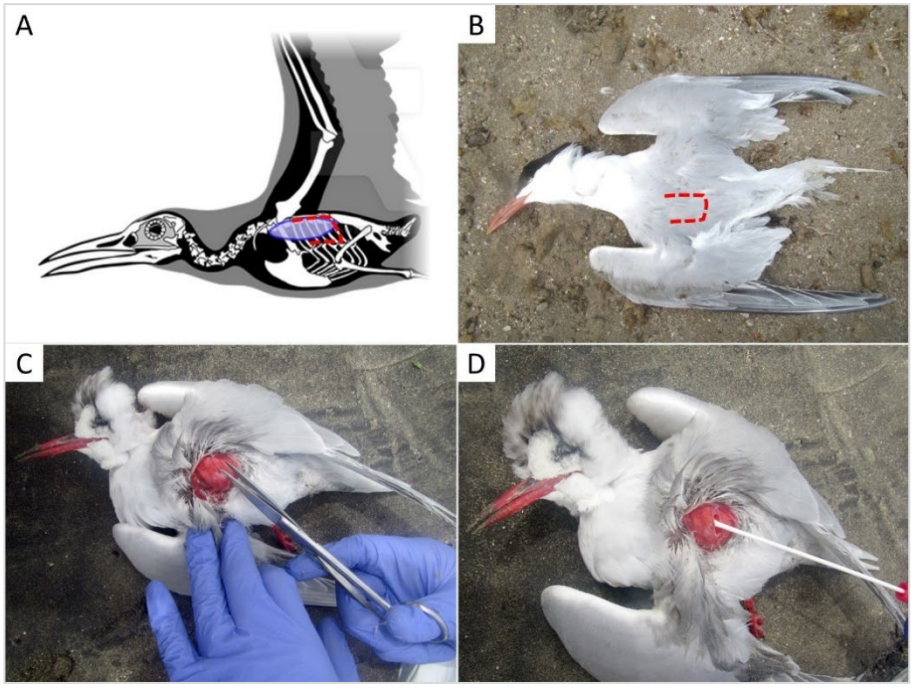


Fig. 6. Collection of swabs from the lungs of birds.

A – Anatomical site where the "window" (red dashed line) should be opened in the interscapular area in order to obtain a swab from the lungs (blue) of birds.
B – Approximate anatomical position of the "window" (red dashed line) to be opened on the torso of birds, with the carcass in ventral decubitus.
C – Scissors are usually the best instrument to open the skin and expose the dorsal ribcage of birds, with the lungs being visible from the outside.
D – After the ribcage is cut with scissors, a swab can be inserted into the lung parenchyma.

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6. Swab from the brain. In pinnipeds and cetaceans, brain swabs can be obtained without opening the skull by inserting a swab through the foramen magnum (Fig. 7A). This is achieved by making a cut to the neck directly behind the end of the skull (Fig. 7B), using the occipital crest as a landmark. Using the knife to separate the skull and the first vertebra (atlanto-occipital joint), the foramen magnum is exposed (Fig. 7C), allowing a swab to be inserted deep into the brain (Fig. 7D). In birds, it is usually easier to expose the brain because the skull is thinner (Fig. 8A), hence a more direct approach is adopted by cutting the skin to expose the skull (Fig. 8B), then the skull is opened with scissors (Fig. 8C) or pruning pliers (for larger species) to expose the brain, allowing swab to be inserted into the brain (Fig. 8D).

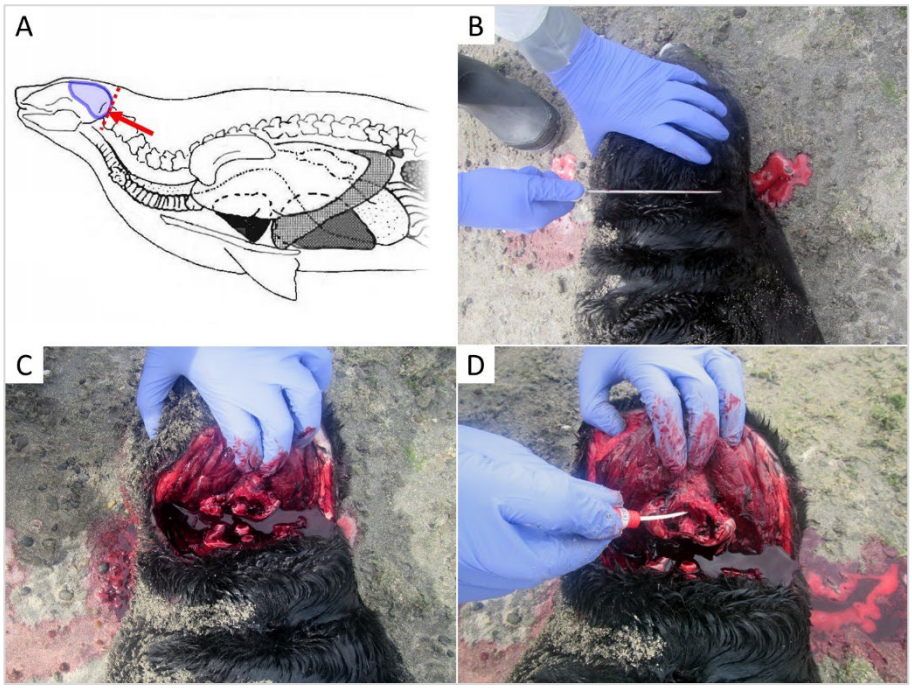


Fig. 7. Collection of swabs from the brain of pinnipeds.

A – Anatomical site where the neck is cut (red dashed line) to access the foramen magnum through which the swab is inserted (red arrow) into the brain (blue).
B – Cutting the neck directly caudal to the occipital crest.
C – The cut should fully separate the skull from the first vertebra (atlanto-occipital joint), exposing the spinal cord.
D – The swab is inserted into the foramen magnum, perforating deep into the brain.

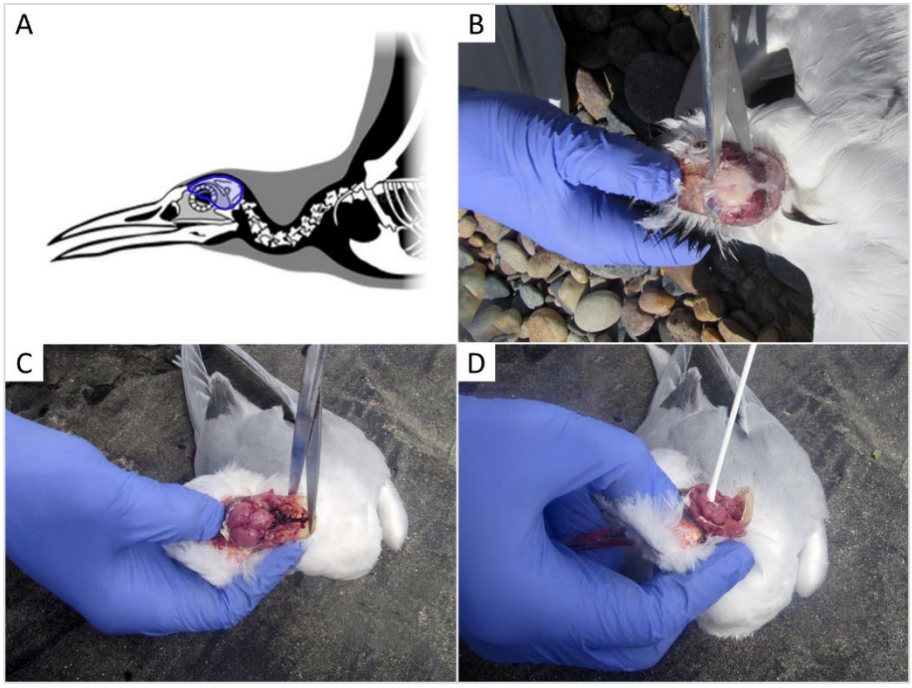


Fig. 8. Collection of swabs from the brain of birds.

A – Anatomical site where the brain (blue) is located in birds.
B – Cutting the skin to expose the skull.
C – In smaller bird species (shorebirds, terns, etc.) the skull can be opened with scissors.
D – Once the brain was exposed, the swab is inserted into the brain parenchyma.

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(Annex 4.3.3)

Additional notes for HPAI sampling for the South African

National Antarctic Program (updated April 2024)

Glossary

antemortem- before death
caudally- towards the tail/ backwards
decubitus - recumbency- lying down
dorsal- back
foramen magnum- opening in the base of the skull
lateral- side
occipital crest- ridge running down the middle of the skull towards the foramen magnum
terminal- end
ventral- underside

Sample numbers

There will be limited consumables available for sampling so they should be used carefully. Three live and three dead individuals of each species will be enough, once the mortality rate and clinical signs have been discussed and concluded to be consistent with HPAI.

Where possible, take a video and consult with an experienced person before sampling a live bird. Otherwise try to make sure that the bird is showing consistently abnormal behaviour, or that there is more than one bird behaving strangely, before sampling.

If an abnormally large number of carcasses is noted, without a visible cause of death, samples can be taken from up to 3 animals.

Personal protective equipment (PPE)

Please see the specifications in section 12.1 of the HPAI protocol

Sample collection and documentation materials

Also take a list of information to be collected about the samples and animal (Annex 3.2.2)

Swabs

Use a separate swab to sample each area or organ.

Up to 2 swabs can be added to each tube of storage medium. The oropharyngeal/ tracheal and cloacal swabs from live birds can be placed together in a tube and the trachea and lung samples from dead animals can be stored together. A brain sample can be stored with a tracheal or lung sample (if only trachea or lung was sampled and not both) otherwise it can be stored separately.

If swabs with breakable handles are not available, it will be necessary to cut the swab handle with side cutters or scissors so that the swab fits into the tube with transport medium. However, the side-cutters/ scissors must be cleaned and disinfected thoroughly between samples to prevent cross-contamination. Wipe with a bleach solution to disinfect, allow at least 10 minutes contact time and ensure that the equipment is as dry as possible before re-use, to avoid depositing bleach in the next tube.

Note: Alcohol does not destroy the viral RNA sufficiently and may still interfere with lab tests so must not be used alone for disinfecting sampling equipment.

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Use of storage medium

- Scrape off excess tissue before inserting the swab in the tube, with the storage medium (supplied)
- A guano sample must not be more than 10% of the fluid volume.
- If a precipitate forms, warm the tube of DNA/RNA Shield or *RNAlater* to just above 37°C (body temperature) and shake to re-dissolve.
- The tubes with storage medium can be kept at room temperature until they are used.

DNA/ RNA Shield:

- Shelf life at room temperature is 60 months (5 years)

RNA*later*:

- Leave the sample in the RNA*later* in the fridge (approx. 4°C) overnight before placing in the freezer.
- Do not allow any mixing with bleach! It is dangerous for you and will damage the RNA.

Sample labelling and record-keeping

Ensure that no two sample tubes can be confused, bearing in mind they may only be defrosted and tested many months later.

Write on the tube:

1. A sample ID code, comprising: species code (see table below) and a number if more than one of that species were sampled on the same day and in the same area (e.g. NGP 01).
   If there are two tubes for one animal (different samples), mark them A and B
   e.g. **NGP 01 A**
2. Date as dd-mmm-yyyy (e.g **02-Jul-2024**)
3. Beach/ colony/ place/ area name or code OR waypoint number

Place the tubes for each animal in a zip-lock bag and write the same details as above on the bag and include a piece of paper in the bag with all sample and animal information, written in pencil (so it will not smudge when damp):

- Sample ID code
  - A: Sample type (e.g. brain swab)
  - B: Sample type (e.g. cloacal + tracheal swabs)
  - C: Sample type
- Date (dd-mmm-yyyy)
- Time (24h)
- Beach code or area name
- GPS coordinates e.g. -46.83456 (S), 37.78965 (E)
- Waypoint number
- Any identifying markers e.g. tag/ring number
- Species (full common name)
- Age class
- Sex (if possible)
- Name of person sampling

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Record the details of the sampled animal in a field notebook, as well as details of other carcasses/ sick animals and other observations and actions. Photograph each page after every sampling effort as back-up, then transfer the data to the Excel sheet at base.

Species	**Code for sample tube**
Birds	
Antarctic Tern	AT
Black-bellied Storm Petrel	BSP
Blue Petrel	BP
Cape (Pintado) Petrel	CP
Common Diving Petrel	CDP
Crozet Shag	CS
Fairy Prion	FP
Gentoo Penguin	GPn
Great-winged Petrel	GWP
Grey Petrel	GP
Grey-backed Storm Petrel	GSP
Grey-headed Albatross	GHA
Indian Yellow-nosed Albatross	IYA
Kelp Gull	KG
Kerguelen Petrel	KP
Kerguelen Tern	KT
King Penguin	KPn
Lesser Sheathbill	LS
Light-mantled Albatross	LMA
Macaroni Penguin	MPn
Northern Giant Petrel	NGP
Salvin's Prion	SP
Soft-plumaged Petrel	SPP
Sooty Albatross	SA
South Georgian Diving Petrel	SDP
Southern Giant Petrel	SGP
Southern Rockhopper Penguin	RPn
Sub-Antarctic (Brown) Skua	SAS
Wandering Albatross	WA
White-chinned Petrel	WCP
Mammals	
Southern Elephant Seal	SES
Sub-Antarctic fur seal	SAFS
Antarctic Fur Seal	AFS

For queries and suggested updates, contact Laura.Roberts@westerncape.gov.za/ +27 72 184 8642

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Marion Island

Recommended HPAI sampling kit supplies

Category	Item	Total	Supplier	Comment/ specifications
Sampling consumables	DNA/ RNA Shield (ZR R1100-250) **OR** RNAlater (AM7020)	500 ml	Inqaba Biotech **OR** ThermoFisher	molecular transport medium- MTM
Sampling consumables	Swabs	1000		rayon-tipped throat swabs with plastic handles
Sampling consumables	Permanent markers	10		Artline EK725 are fine enough for small tubes
Sampling equipment	Surgical scissors			Stainless steel. Preferably straight with sharp tips (sharp-sharp). E.g Metzenbaum dissecting scissors
Sampling equipment	Surgical forceps			Stainless steel. Preferably rat-toothed
Sampling equipment	Scalpel handle			Stainless steel. Preferably size 24.
Sampling equipment	Scalpel blades			Preferably size 24.
Sampling equipment	Butchers knife			for seals- 15-25cm
Sampling equipment	Pencils (& sharpener)			
Sampling equipment	Zip-lock bags			
Sampling equipment	Notebook			
PPE	Gumboots	1/person		
PPE	Goggles	1/person		seal onto the face, E.g. http://www.northsafety.co.za/product/Eye+Protection#id=377
PPE	Disposable, fluid-resistant suits	100	North Safety/ Dromex	equivalent to European standard EN 14126, CE certified as Category III, Type 5-B or 6-B (EN 13982-1:2004 or EN 13034:2005) E.g. Tyvek 500 suit https://www.dupont.com/products/dupont-tyvek-500-ty198s-wh.html
PPE	Respirators	100		equivalent to EN149 FFP2 e.g. http://northsafety.co.za/product/Respiratory+Protection/RPD1+SH3720 or NIOSH N95 or KN95 GB2626-2006 accredited
PPE	Disposable latex or nitrile gloves	600		2 boxes each of small, medium & large
PPE	Duct tape			
Disinfection	F10 SC	5L	Health and Hygiene	
Disinfection	Jik	2L		plain bleach product without other additives (ingredients only sodium hypochlorite, preferably no scent)
Disinfection	spray bottles, 500ml	5		
Disinfection	Large scrubbing brushes	5		

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Marion Island HPAI Protocol

Annex 4.3.5- Draft Media Statement

[Date]

High pathogenicity Avian influenza suspected on Marion Island

High pathogenicity avian influenza (HPAI) virus is suspected to have reached Marion Island, one of South Africa's sub-Antarctic islands in the southern Indian Ocean. In recent weeks deaths in [species] have increased and some birds have shown signs indicative of the disease. To date [numbers of each species] are estimated to have died. The virus can be transported long distances by migrating birds, and this is likely how the virus arrived on the island.

[Background]

Having spread across the globe since 2021, HPAI (H5N1) was detected in seabirds and marine mammals on the island of South Georgia, southeast of South America, in October 2023. It was detected in Antarctica in February 2024 and has since [any updates on other sites affected]. The Prince Edward Islands, comprising Marion Island and Prince Edward Island, host millions of seabirds, including half of the world's wandering albatrosses and hundreds of thousands of penguins, and is also home to a major colony of Sub-Antarctic Fur Seals. An HPAI outbreak is therefore of extreme conservation concern.

[Allay fears/ reassure about action being taken/ stress role-players involved]

It is unfortunately impossible to confirm the presence of the virus at this time, as there will be no transport available for samples until next April. However, the event has met previously-decided criteria in order to be classified as a likely HPAI event. The situation is being closely monitored by the eleven personnel over-wintering on the island who have been trained to recognise possible HPAI signs in birds and seals, and in the necessary monitoring and mitigation methods.. They will be taking all precautions to ensure they do not spread the virus, and are collecting information to assist with decisions about targeted mitigation.

The South African National Antarctic Program will continue to work with the Department of Forestry, Fisheries and Environment, Western Cape Veterinary Services, marine ornithologists, marine mammal biologists disease experts and colleagues overseas with similar experience, to monitor and combat the spread of the virus.

-ENDS-

MEDIA ENQUIRIES:

[Organisation]

Communications Officer

[Name]

[email address]

[Telephone number

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Annex 5: Permits and Approvals

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Permits and approvals for Chapter 4: Survey of HPAI in African Penguins

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Annex 5

UNIVERSITEIT VAN PRETORIA
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Faculty of Veterinary Science

Animal Ethics Committee

Approval Certificate with Condition
New Application

AEC Reference No.: V007-19
Title: Avian flu survey of African penguins
Researcher: Prof D Abernethy

15 April 2019

Dear Prof Abernethy

This new application for your research received in January 2019, was approved *ad hoc* with the following conditions:

1) The study cannot start until the permit is received and a copy made available
2) If the sampling of the rehab birds is to go ahead, the committee needs to be informed.

Please note the following about your ethics approval:

1. The use of 1136 African penguin (*Spheniscus demersus*) species is approved.
2. Ethics Approval is valid for 1 year and needs to be renewed annually by March 2020.
3. Please remember to use your protocol number (V007-19) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Room 6-2, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8483
Fax +27 12 529 8321
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www.up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

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agriculture, land reform & rural development
Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Land Reform & Rural Development
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@dalrrd.gov.za
Reference: 12/11/1//1/6/ 1700/MG

Prof Darell Abernethy
C/O Centre for Veterinary Wildlife Studies
Faculty of Veterinary Sciences
University of Pretoria
Onderstepoort
0100
E-mail:Daa47@aber.ac.uk

Dear Prof Darell Abernethy,

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Your application received per email, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. The study is approved as per the application form dated 08 July 2020 and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this study under this Section 20 permit. Please apply in writing to HerryG@dalrrd.gov.za;
3. All potentially infectious material utilised, collected or generated during the study are to be destroyed at the completion of the study. A registered waste removal company must dispose the material generated from the study Records must be kept for five years for auditing purposes;

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4. Blood, tracheal and cloacal samples collected from penguins in the Western Cape province should be send to Western Cape Provincial Laboratory for Avian Influenza testing. If AIV RNA is detected it must be sent to the BSL3 Poultry laboratory at the University of Pretoria for sequencing;
5. At the end of the study, excess serum and prepared blood smears samples and swab transport medium must be send for storage at the Companion Animal Studies Laboratory Biobank at the University of Pretoria;
6. Any further use or distribution of the bio banked samples is subject to obtaining a separate Section 20 approval;
7. If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 approval.

Title of research/study: Survey for avian influenza virus in African penguins:

Researcher:	Prof Darell Abernethy,
Institution:	University of Pretoria, Centre for Wildlife Studies.
Our ref Number:	12/11/1/1/MG
Your ref:	V007-19
Expiry date:	31 December 2023

Kind regards,

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2021 -02- 23

SUBJECT: Survey for avian influenza virus in African penguins:

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environment, forestry & fisheries
Department:
Environment, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Enquiries: L Swart (permit administrator)
Tel: 021-819 5056
E-mail :researchpermits@environment.gov.za

Centre for Veterinary Wildlife Studies
Faculty of Veterinary Science
University of Pretoria
Private Bag X04
Onderstepoort
Pretoria
0110

Attention: Dear Dr Laura Roberts

PERMIT FOR THE PURPOSES OF A SCIENTIFIC INVESTIGATION OR PRACTICAL EXPERIMENT IN TERMS OF SECTION 83 OF THE MARINE LIVING RESOURCES ACT, 1998 (ACT NO. 18 OF 1998).

I, the undersigned, Director: Biodiversity and Coastal Research, Branch Oceans and Coasts, Department of Environment, Forestry and Fisheries(DEFF) (the Director) acting in pursuance of the delegated authority conferred upon me by the Honourable Minister of Environment, Forestry and Fisheries as contemplated in terms of Section 79 of the Marine Living Resources Act of 1998 (Act No. 18 of 1998) ("the Act") hereby permit, in terms of Section 83 of the Act, the following person(s)/institution to engage in the scientific investigation or practical experiment referred to below:

PERMIT REFERENCE NUMBER: Res2020-54
PERSON(S): Dr Laura Roberts
INSTITUTION: University of Pretoria
SCIENTIFIC INVESTIGATION OR PRACTICAL EXPERIMENT: to do a comprehensive diseases survey along the entire breeding range of the African penguin for the purposes of research,

subject to the following conditions:

1. GENERAL CONDITIONS

1.1. This permit is issued subject to the provisions and regulations of the following laws:
(a) The Marine Living Resources Act, 1998 (Act No. 18 of 1998) ("the Act"), and all regulations published in terms thereof;
(b) The National Environmental Management Act, 1998 (Act No. 107 of 1998) ("NEMA"), and in particular, the regulations that control vehicle use in the coastal zone (as

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amended);
(c) The National Environmental Management Biodiversity Act, 2004 (Act No. 10 of 2004);
(d) The National Environmental Management Protected Areas Act, 2003 (Act No. 57 of 2003);
(e) The Sea Birds and Seals Protection Act, 1973 (Act No. 46 of 1973); and
(f) Prevention of Pollution from Ships Act (Act No. 2 of 1986).
(g) National Environmental Management Integrated Coastal Management Act, 2008 (Act No. 24 of 2008)
(h) Any other relevant law
(i) All relevant COVIT 19 regulations, as published in the Government Gazette, should be adhered to during the period of the pandemic.
(j) All relevant COVIT 19 regulations by SAMSA, for the use of ships and boats should be adhered to during the period of the pandemic.

1.2. This permit is intended to facilitate *bona fide* research, and should not be used for the purposes of financial gain by the Permit Holder.

1.3. If, in the opinion of the Chief Director or Director there are sound reasons for doing so, the Chief Director or Director may amend the relevant conditions of the permit.

1.4. Any reference to the Permit Holder in these permit conditions includes the entity or person, his/her or its employees (whether permanent, full-time or part-time), his/her or its contractors, agents or advisers, being cognisant of the course and scope of their contractual relationship.

1.5. A breach of the provisions of the Act, regulations or these permit conditions by the Permit Holder may result in the initiation of legal proceedings (civil or criminal). A breach includes:

(a) furnishing information to which the Department of Environment, Forestry and Fisheries (the Department) is entitled, which is not true or complete;

(b) contravening or failing to comply with a permit condition or with the provisions of the relevant legislation in Section 1.1.

(c) being convicted of an offence in terms of the relevant legislation in Section 1.1.; or

(d) failing to effectively utilise the permit.

1.6. The Permit Holder shall store at their registered place of business/residence the original permit issued. The Permit Holder shall at all times, have available a true certified copy of this permit which should be produced on demand by any law enforcement official.

1.7. This permit shall only be utilized by the individual/organisation whose name appears on the permit.

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1.8. If the permit is in the name of an institution/company/close corporation the individual utilizing the permit shall in addition to a certified copy of this permit, be in possession of identification and/or letter which identifies the individual as an authorized person or employee of the permit holder.

1.9. Any individual utilizing this permit shall in addition to the above conditions have a certified copy of any other permit/exemption required in terms of other legislation including any permit or exemption in terms of the Marine Living Resources Act, 1998 (Act No. 18 of 1998).

1.10. An application for a future permit may be refused if the conditions of this permit are not adhered to.

1.11. In terms of the Marine Living Resources Act, the Permit Holder is obliged to report to the Minister any contravention of the provisions of the MLRA by any other person.

1.12. The Permit Holder must safely store all inorganic waste material, garbage and pollutants on board the vessel or at the site of research activities if on land. Should the Permit Holder discard any inorganic waste material, garbage or pollutants into the sea or coastal environment, the Department may institute legal proceedings (civil or criminal) which may include suspension of the permit for a period determined by the Department and the Permit Holder shall take those steps considered necessary in terms of relevant legislation to remedy any pollution caused.

1.13. This permit does not in any way absolve the Permit Holder from the obligations of and adhering to the remainder of the provisions and conditions of the MLRA, regulations or any other law.

1.14. Any installations must be removed on termination of the project(s).

1.15. Specimens collected in terms of this permit shall not be sold or offered for sale.

1.16. This permit may not be used in marketing materials of the Permit Holders, or in any way to contract business to the Permit Holder.

1.17. No harmful chemicals are to be used when collecting marine species. Limited use of fish anaesthetics (including rotenone) is permitted if no other suitable technique is available to collect fishes, and should be kept to a minimum. Local authorities should be advised when rotenone is to be used to collect fish.

1.18. No vehicle may be used in the coastal zone in terms of this permit unless the permit holder is in possession of a valid permit to use a vehicle in the coastal zone in terms of the Regulations for the Control of Use of Vehicles in the Coastal Zone (GNR 1426 of 7 December 2004).7

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1.19. Reports as stipulated in the 'Specific Conditions' must be submitted to The Directorate: Biodiversity and Coastal Research, Attention: L Swart by email to researchpermits@environment.gov.za. Such reports must be submitted within the timeframes provided, before any renewal of this permit or application for any other permit will be considered.

1.20. The report, as required under Condition 1.19, should provide details of the dates, locations, species and quantities collected on the template provided by the Department.

1.21. This permit may not be utilised within a marine protected area for collecting, monitoring or any other activity which is prohibited in terms of section 43 of the Act and regulations unless such activity is specifically authorised in the section 2 of this permit.

2. SPECIFIC CONDITIONS FOR MARINE PROTECTED AREAS/HABITATS AND PROTECTED SPECIES

2.1. This permit allows for the comprehensive diseases survey along the entire breeding range of the African penguin for *bona fide* research projects of Dr Laura Roberts of the Centre for Veterinary Wildlife Studies, Faculty of Veterinary Science at University of Pretoria.

2.2. The permit will allow the sampling of 450 individual African penguins which should be spread across the breeding localities

2.3. Smaller breeding colonies with less than 100 pairs should be avoided as any handling may cause them to abandon the eggs and thereby breeding failure.

2.4. To prevent undue stress, handling times should not exceed ten minutes from capture.

2.5. If the birds move away from the eggs or small chicks, an effort must be made to cover the nest contents with nesting cryptic materials and then move away.

2.6. Research co-ordination between other researchers (Drs Sherley, McIness, Pichegru, Ludynia, Connan and Makhado) is encouraged.

2.7. No duplication of research activities will be encouraged especially when dealing with an endangered species such as African penguins.

2.8. No additional sample size will be approved.

2.9. When visiting any seabird colony, due care must be taken not unduly to stress any seabird or cause the abandonment of any breeding attempt. Minimal disturbance should be adhered to.

2.10. All mitigation measures specified in the project application must be implemented.

2.11. Any visits to or activities conducted at African penguin breeding colonies may only

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be undertaken once approval in writing has been obtained from the authority that manages the colony. The authority must be informed well in advance of any visit/work to be undertaken.

2.12. Any research activity on any seabirds is prohibited without ethics clearance and the permit holder must seek ethical clearance or approval for conducting such research activities. Failure to do that, precludes such activities from being undertaken.

2.13 The detailed bio-security protocols that will be followed must be provided and must be complied to avoid spread of avian diseases between colonies. Details of this should be included in the progress report of permit condition in 2.17

2.14. No mammals, turtles, other species of marine birds or any protected marine species may be collected or disturbed.

2.15. This permit does not allow for any specimens to be used in any way in a bioprospecting context

2.16 This permit does allow collection within the specifically mentioned Marine Protected Areas only.

2.17. The Permit Holder must submit a progress report to researchpermits@environment.gov.za by end October 2020.

3. PERMIT VALIDITY PERIOD

This permit is valid from **date of issue** until **31 December 2020.**



GERHARD CILLIERS
DIRECTOR: BIODIVERSITY AND COASTAL RESEARCH
BRANCH: OCEANS AND COASTS
DATE: 25/06/2020

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Permit Number: CRC/2020-2021/022--2019/V1
CAPE RESEARCH CENTRE

P.O. Box 216, STEENBERG, 7947
Tel: +27 (0)21 713 7511; Fax: +27 (0)21 712 0131

Research Permit: TABLE MOUNTAIN, WEST COAST AND ADDO ELEPHANT NATIONAL PARKS

12 August 2020

Dr Darrell Abernethy – "Epidemiology and management of highly pathogenic avian influenza in African penguins in SANParks-controlled colonies"

Co-workers: Alison Kock, Katta Ludynia, Lauren Waller, Nola Parsons, Celia Abolnik, David Roberts, Laura Roberts, REJ Burroughs, Romy Klusener, France van Wyk, Curtly Ambrose, Melissa Cadman, Peter van der Linde, Kyle Maurer, Wellington Andile, Albert Snyman, University of Cape Town, Department of Biological Sciences

The permit is valid from **12 August 2020** until **31 August 2021** and subject to the following conditions. The Park Management staff must be contacted prior to entry into the park (see list of staff members below). You are allowed access to **the boulders section** of the park.

Standard Conditions:

• The use of non-demarcated areas will lead to the disturbance of animals and eco-systems, trampling of vegetation and soil erosion and only the use of accepted pathways and areas is therefore permitted, UNLESS BY SPECIAL ARRANGEMENTS. PLEASE CONTACT THE PARK MANAGEMENT STAFF IF RESTRICTED AREAS NEED TO BE ACCESSED.
• No damage shall be permitted to any natural vegetation, environment or property.
• Animals may not be disturbed in any way.
• Uncontrolled vehicle access and parking could cause damage to vegetation and soil erosion and therefore only the use of approved vehicles routes and parking areas is allowed.
• Fires can cause loss of vegetation, soil erosion and life and therefore fires, and braai's are not permitted unless in dedicated braai areas.
• Remove all rubbish and waste as it has an effect on the health of visitors, animals and plants.
• Other visitors to the area and or neighbours may not be hindered in any way.
• Pollution affects the health and safety of animals, plants, visitors and neighbours and is not permitted.
• Excessive noise affects animals (e.g. birds nesting in the areas), visitors and or neighbours and is not permitted.
• Your permit and personal identification must be retained and kept on your person at all times, and produced on request.
• The areas under the control of SANParks are used entirely at your own risk. South African National Parks, its Board, directors, employees and agents are not liable for any loss or damage to the property or possession of any guest or participant (or accompanying minor) whether such damage is caused by the negligent act or omission of South African National Parks; arising from death or any bodily injuries of whatsoever nature sustained by a guest or participant (or accompanying minor) whether such injuries are caused by the negligent act or omission by South African National Parks, and/or by the defective functioning of any apparatus. The guest or participant and/or his/her/their estate hereby indemnifies South African National Parks against any claim, action, judgment, costs and/or expenses which may be made against South African National Parks and as may in any way be related to the above. The onus lies with the company or applicant to ensure that they are adequately insured.
• Please note that you (your staff etc) are subject to the conditions of Section 86 of the National Environmental Management Act (107 of 1998) and the National Environmental Act: Protected Areas Act (Act 57 of 2003) and any other relevant legislation for the duration of your stay in the National Park (e.g. adherence to the Registrar's requirements for guides).

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• SANParks staff's instructions, notices, regulations and signs must be complied with.
• The activity shall be restricted to the area applied for.
• Gate and operating times to be complied with.
• No pets allowed.

Special Conditions:

- Researchers need to co-ordinate field work with existing research and monitoring in each colony (Drs McInnes, Pichegru, Ludynia, and Makhado and the penguin rangers / monitors)
- Only experienced penguin handlers to minimise capture and handling stress
- Max 3 people on the sampling team at one time
- Follow all biosecurity protocols as per the addendum in the proposal
- When visiting any seabird colony, due care must be taken not unduly to stress any seabird or cause the abandonment of any breeding attempt.
- To prevent undue stress, handling times should not exceed ten minutes from capture
- The following biological collections have been approved for 1200 penguins across all sites:
    - 20ml blood from jugular
    - 6 feathers
    - Opportunistic endoparasites, ectoparasites and cloacal/pharyngotracheal swabs may be collected

Park	Park Management Staff	Telephone Number:
Cape Research Centre	Alison Kock Alison.Kock@sanparks.org	021 713 7510
TMNP - Boulders	Faroeshka Rodgers Faroeshka.Gool@sanparks.org	
WCNP	William Brink william.brink@sanparks.org	
AENP	Cathy Dreyer Catherine.Dreyer@sanparks.org	

Yours faithfully,



Debbi Winterton, Science Liaison Officer; E-mail: deborah.winterton@sanparks.org

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Annex 5

Telephone No: (027) 021 483 0000
E-mail: permits.fax@capenature.co.za
PGWC Shared Services Centre
cnr Bosduif and Volstruis Streets
Bridgetown
7764

Facsimile No: (027) 0865567734
Internet: www.capenature.co.za
Private Bag X29
Gatesville
7766

PERMIT TO
HUNT WITH PROHIBITED HUNTING METHOD OF WILD ANIMALS – RESEARCH PURPOSES

Issued in terms of the provisions of the Nature Conservation Ordinance 1974, (Ord 19 of 1974)(Section 29 & 33)
Not Transferable

HOLDER

Full Name:	Dr. Laura Christl Roberts	**Identity No:**	8807020096087
Trade Name:	University of Pretoria	**Registration No:**	
Postal Address	Private Bag X04	**Physical Address:**	1 Soutpan Rd (M35)
City / Town:	Onderstepoort	**City / Town:**	Onderstepoort
Province / State:	Gauteng	**Province / State:**	Gauteng
Country:	South Africa	**Country:**	South Africa
Postal / Zip Code:	0110	**Longitude:**	
		Latitude:	

In terms of and to the provisions of the abovementioned Ordinance and the Regulations framed thereunder, the holder of this permit is hereby authorised to Hunt (capture/disturb/stampede/kill) the protected wild animal(s) specified below on the property mentioned on this permit. See conditions on last page.

DETAILS

Permit / License No:	**CN44-59-13038**
Expiry Date:	**31/12/2021**
Date Issued:	08/04/2020
Amount Paid:	R 0.00
Reference:	
File Code:	1/2/1/6/5/F21

Stamp: CapeNature FAUNA • FLORA • HUNTING • CITES

DESCRIPTION — PROPERTY

Organization	University of Pretoria
Full Name:	Dr. Laura Christl Roberts
Identity Number:	8807020096087
Postal Address	See special conditions for details
City / Town:	NA
Province / State:	Western Cape
Country:	South Africa
Postal / Zip Code:	0000
Longitude:	
Latitude:	

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SPECIES (SCIENTIFIC NAME)			NOTE
African Penguin	(Spheniscus demersus)	70	Maximum at Dassen Island. For sampling and marking purposes. No collection.
African Penguin	(Spheniscus demersus)	32	Maximum at Dyer Island. For sampling and marking purposes. No collection.
African Penguin	(Spheniscus demersus)	80	Maximum at Stony Point. For sampling and marking purposes. No collection.
Note.	(Note.)	0	A tracheal and cloaca sample will be collected from all birds by quickly wiping a cotton swab at the tracheal opening and then inserting quickly into the vent.
Note.	(Note.)	0	Conditions apply. Note special conditions.
Note.	(Note.)	0	Method: By hand. See special conditions for details.

F. Arnold 08/04/2020

Issued By:	Approved on Behalf CEO	Effective Date:	Signature of Holder:
Fadwa Arnold	Western CapeNature Conservation Board		I acknowledge, accept and understand fully the permit conditions as described.

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Annex 5

1. When the holder of this permit *kills/captures/collect any wild animal in terms thereof, he shall, before leaving the abovementioned property, or if he does not leave it, after each day's hunt/capture/collection, record the particulars regarding the date, species and number of each sex of each species, or if it is impossible to distinguish the sex, the total number of each species of such wild animals which he had *killed/capture/collected.
2. The holder of this permit shall return it to the Chief Executive Officer: Western Cape Nature Conservation Board, Private Bag X29, Gatesville, 7766, within 14 days of the date of expiry thereof.
3. THIS PERMIT IS SUBJECT TO THE SPECIAL CONDITIONS AS SET OUT IN THE ADDENDUM HERETO.

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Annex 5

TITLE OF PROJECT: Epidemiology of highly pathogenic avian influenza in African penguins

COLLECTION AREAS: Within the Western Cape Province including Stony Point Nature Reserve, Dyer and Dassen Island.

METHOD:

a) Penguins will be caught either individually, when sitting on their nests or by herding non-breeders into a group and placing them in SANCCOB's specially designed holding boxes until they are processed. Birds will be captured by local, experienced rangers, processed and immediately returned to their nests.

b) The bill length and depth as well as head length will be measured and recorded and the bird will be weighed, thus the birds' overall condition will be evaluated.

c) Blood (15 – 20 ml) will be collected, by trained veterinarians or paraveterinarians, from the jugular vein in the neck. Birds will be visually inspected for ectoparasites, which will be collected and placed in alcohol. Faeces will be opportunistically collected for endoparasite examination and microbiome analysis.

d) A PIT, if not already present, will be inserted subcutaneously between the left leg and the tail and six feathers will be plucked for DNA sexing and stable isotope work, according to the permitted procedure of inserting the microchip. The bird will then be returned to the colony or its nest.

e) Blood smears will be made at the end of the sampling day from collected blood for haemoparasite identification and to detect genetic material of pathogens.

THIS PERMIT IS ISSUED SUBJECT TO THE FOLLOWING SPECIAL CONDITIONS:

1. This project must be done in collaboration with the PIT tag program.
2. An annual report is required on the number of birds that have been tested and must also indicate any difficulties or issues experienced so that CapeNature can learn from them for future studies.
3. The numbers of birds to be sampled at each colony managed by CapeNature is restricted to the following: Dassen Island 70, Dyer Island 32, and Stony Point 80.
4. This permit is relevant for research done on CapeNature properties. Where research is done on municipal, private or other properties, permission and/ or permits must be obtained separately from them.
5. Copies of all reports or publications emanating from this research must be forwarded to CapeNature (avlok@capenature.co.za) for dissemination to relevant staff.
6. The data of all species collected must be captured on the SOB datasheet and submitted electronically to CapeNature (avlok@capenature.co.za) for inclusion in the SOB database.
7. The Conservation Manager has the right to refuse access to sensitive areas.
8. The relevant conservation manager must be contacted at least 2 weeks in advance to discuss and agree on logistical arrangements for the field work.
9. Please keep Kevin Shaw (shawk@capenature.co.za) and Alexis Olds (aolds@capenature.co.za) informed of project progress and visits to colonies managed by CapeNature.

PERSONS ENGAGED IN THIS PROJECT:

1. Prof. D Abernethy; Researcher, Centre for Vet.Wildlife Studies; Faculty of Veterinary Science,University of Pretoria; P.I.; Veterinary epidemiologist; Project design and coordination
2. Prof. C Abolnik; SARCHI Research Chair in Poultry Health and Production; Faculty of Veterinary Science,University of Pretoria; Virologist. HPAI diagnosis;
3. Dr R. Burroughs; Wildlife expert, Dept.Production Animal Studies; Faculty of Veterinary Science,University of Pretoria; Clinician. Field sampling and project coordination.
4. Prof. C Donnelly Professor, Department of Statistics.Oxford University, United Kingdom Epidemiologist; project design and data analysis.
5. Dr J. Kemper Research Associate African Penguin Conservation Project, Namibia Ecologist; Field sampling
6. Dr K. Ludynia Research Manager South African Foundation for the Conservation of Coastal Birds (SANCCOB) Ecologist; Field sampling
7. Prof M. Oosthuizen Deputy Dean Faculty of Veterinary Science, University of Pretoria; Microbiologist; Microbiome analysis
8. Dr N. Parson Research Associate and Veterinarian SANCCOB Veterinarian. Field sampling
9. Dr L. Roberts State Veterinarian and Epidemiologist Western Cape Dept. Agriculture, Vet Services; Veterinarian. Planning and field sampling; biosecurity
10. Dr D. Roberts Veterinarian SANCCOB Veterinarian. Field sampling
11. Dr J.P. Roux Marine ecologist, Ecosystem Modeller; Ministry of Fisheries and Marine Resources, Namibia; Field sampling, logistic assistance
12. Dr A. Spickett Researcher, Helminthology Onderstepoort Veterinary Research. Helminthologist; Endoparasite identification
13. Dr M. Umberto; Senior Lecturer School of Veterinary Medicine, University of Namibia Molecular biologist; HPAI diagnosis
14. Dr L Waller; Leiden Conservation; Fellow SANCCOB Ecologist; field sampling.

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UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

CONDITIONS APPLICABLE TO RESEARCHERS UNDERTAKING RESEARCH OR OTHER COLLECTING WORKS ON PROVINCIAL CONSERVATION AREAS AND / OR PRIVATELY OWNED LAND IN THE PROVINCE OF WESTERN CAPE:

1. THE MANAGER OF THE RELEVANT CONSERVATION AREA(S) (IF ANY) MUST BE INFORMED TIMEOUSLY BEFORE ANY CONSERVATION AREA IS ENTERED FOR COLLECTING OR RESEARCH PURPOSES AND THE MANAGER'S WRITTEN PERMISSION TO ENTER SUCH RESERVE MUST BE ACQUIRED BEFOREHAND. THIS PERMIT DOES NOT GRANT THE PERMIT HOLDER AUTOMATIC ACCESS TO ANY NATURE RESERVE, CONSERVATION AREA, WILDERNESS AREA AND / OR STATE FOREST. ANY OTHER / FURTHER CONDITIONS OR RESTRICTIONS THAT THE MANAGER MAY STIPULATE AT HIS / HER DISCRETION MUST ALSO BE ADHERED TO. THIS PERMIT MUST BE AVAILABLE TO BE SHOWN ON DEMAND.
2. The owner of any other land concerned (be it privately or publicly owned land) must give WRITTEN consent allowing the permit holder to enter said property to collect flora / fauna. This written permission must reflect the full name and address of the property owner (or of the person authorised to grant such permission), the full name and address of the person to whom the permission is granted and the number and species of the flora / fauna, the date or dates on which such flora / fauna may be picked / collected and the land in respect of which permission is granted. Copies of this written permission must be made available to The Western Cape Nature Conservation Board upon request.
3. Type-specimens of any newly described / discovered species or other taxon collected must be lodged with a recognised South African scientific institution / museum / herbarium (preferably within the Province of Western Cape) where such material will be available to other researchers. For every flora specimen collected on a Western Cape Nature Conservation Board nature reserve, one additional (extra) herbarium specimen must be forwarded to the Western Cape Nature Conservation Board Herbarium at Jonkershoek (c/o MJ Simpson, Private Bag X5014, Stellenbosch 7599).
4. A list of all collected specimens / material including the; species name, the number collected, the collection date and the precise locality of the collection must be submitted within 14 days from the date of expiry of your permit to The Chief Executive Officer: CapeNature, Private Bag X29, Gatesvilles, 7766
5. The maximum number of specimens per species specified in the permit (if at all) may not be exceeded without the prior permission of The Chief Executive Officer: Western Cape Nature Conservation Board.
6. For projects of more than one year's duration a progress report must be submitted to The Chief Executive Officer: Western Cape Nature Conservation Board before 31 December of each year.
7. One copy of all completed reports, publications, or articles (including books, videos, CDs, DVDs etc.) resulting from the project/collection must be submitted to The Chief Executive Officer: Western Cape Nature Conservation Board free of charge.
8. Should a report, publication, article or thesis arise from this project/collection, an acknowledgement to Western Cape Nature Conservation Board must be included.
9. The Forest Act 1984 (Act 122 of 1984) and regulations, the Nature Conservation Ordinance, 1974 (Ordinance 19 of 1974) and all regulations in terms of the Ordinance must be adhered to.
10. Should it be envisaged to export any material / specimens across the boundaries of the Western Cape Province, an export permit will be required in respect of certain species and a further application form will have to be completed. The permit holder must confirm with the Western Cape Nature Conservation Board whether an export permit is required BEFORE exporting any material / specimens from the Western Cape Province.
11. No species that appear on the Red Data List or species listed as endangered in terms of the Nature Conservation Ordinance, 1974 (Ordinance 19 of 1974) may be collected, except for those mentioned on the permit.
12. Unless otherwise specifically indicated in writing, no material or specimens collected with this permit or material or specimens bred or propagated, from material or specimens collected with this permit, may be donated, sold or used for any commercial purpose by any party.
13. IF APPLICABLE, ETHICS CLEARANCE MUST BE ACQUIRED FROM YOUR RESEARCH INSTITUTE PRIOR TO COLLECTION.

CHIEF EXECUTIVE OFFICER
WCNCB

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Permits and approvals for Chapter 5: Vaccination of African penguins against HPAI

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Faculty of Veterinary Science

Research Ethics Committee 10 March 2020

CONDITIONALLY APPROVAL

Ethics Reference No	**REC256-19**
Protocol Title	**Vaccination of African penguins (Spheniscus demersus) against clade 2.3.4.4 H5 highly pathogenic avian influenza (HPAI)**
Principal Investigator	**Dr LC Roberts**
Supervisors	**Prof DA Abernethy**

Dear Dr LC Roberts,

We are pleased to inform you that your submission has been conditionally approved by the Faculty of Veterinary Sciences Research Ethics committee, subject to other relevant approvals.

Please note the following about your ethics approval:

1. Please use your reference number (REC256-19) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application for post graduate studies (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. **Applications using Animals:** FVS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

Notes: Conditionally approved (pending obtaining all other relevant approvals).

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

Room 6-6, Arnold Theiler Building
University of Pretoria, Faculty of Veterinary Science
Private Bag X04, Onderstepoort, 0110, South Africa
Tel +27 (0)12 529 8390
Email marie.watson-kriek@up.ac.za
www.up.ac.za

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Annex 5



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UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

12 June 2020

Approval Certificate
New Application

AEC Reference No.:	REC256-19
Title:	Vaccination of African penguins (Spheniscus demersus) against clade 2.3.4.4 H5 highly pathogenic avian influenza (HPAI)
Researcher:	Dr LC Roberts
Student's Supervisor:	Prof DA Abernethy

Dear Dr LC Roberts,

The **New Application** as supported by documents received between 2020-02-04 and 2020-06-05 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-06-05.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	**Number**
African penguin (Spheniscus demersus) SANCCOB	24
Samples Serum	240 (5 ml each) 10 samples per animal

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-06-12.

3. Please remember to use your protocol number (REC256-19) on any documents or correspondence with the AEC regarding your research.

4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.
Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8483
Fax +27 12 529 8321
Email aec@up.ac.za
www.up.ac.za

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Annex 5

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Name, Department of Agriculture, Forestry and Fisheries
Private Bag X250, Pretoria 0001

Enquiries: Mr Herry Gololo · Tel: 012 319 7532 · Fax: +27 12 319 7470 E-mail: HerryG@daff.gov.za
Reference: 12/11/1/7

Dr Laura Christi Roberts
Department of Agriculture, Main Building
Elsenburg
Western Cape
7607

RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Dear Dr Roberts

Your fax / memo / letter/ Email dated 31 January 2020, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers.

I am pleased to inform you that permission is hereby granted to perform the following research/study, with the following conditions :

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study. Records must be kept for five years for audit purposes. A dispensation application may be made to the Director Animal Health in the event that any of the above is to be stored or distributed;
3. This Section 20 approval is issued based on:
   i) the clinical trial approval issued by SAHPRA with SAHPRA Approval Number: VCT 08/2020 indicating that the applicant has addressed all the safety matters; and

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ii) The permission for use of unregistered products issued in terms of Section 21 of Act 101 of 1965 with authorisation number VCT/08/2020;

4. Ethics approval must be obtained prior to the start of the study;
5. The only vaccines that may be used for this study are the plant-derived H5 avian influenza VLP vaccine produced by the CISR and the inactivated H5N8 HPAI whole virus vaccine produced by Deltamune;
6. No challenge may be conducted as part of this study;
7. The only penguins that may be vaccinated are resident penguins housed at the South African Foundation for the Conservation of Coastal Birds (SANCCOB) in Table View;
8. The microchips of the penguins that are vaccinated at SANCCOB must be recorded as these vaccinated penguins must remain at the SANCCOB facility for the rest of their lives and may not be released;
9. Any remaining plant-derived H5 avian influenza VLP vaccine and inactivated H5N8 HPAI whole virus vaccine at the end of the study must be destroyed;
10. Remaining penguin sera at the end of the study may be transported from the WCPVL to the Poultry Research Laboratory, Faculty of Veterinary Science, University of Pretoria for storage;
11. This Section 20 approval is valid until 30 April 2022.

Title of research/study: Vaccination of African penguins (*Spheniscus demersus*) against clade 2.3.4.4 H5 highly pathogenic avian influenza (HPAI)
Researcher (s): Dr Roberts
Institution: Department of Agriculture, Western Cape; SANCCOB
Your Ref./ Project Number: REC256-19
Our ref Number: 12/11/1/7

Kind regards,

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2020 -06- 01

SUBJECT: **RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 of 1984)**

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SAHPRA
South African Health Products Regulatory Authority

CSIR Campus
Building 10F
Meiring Naudé Road
Brummeria
Pretoria

Enquiries: Dr A.T Sigobodhla
References: Rec256-19
19 May 2020

Department of Agriculture
Elsenburg, Muldersvlei Road
WESTERN CAPE
7607

Att: Dr L. Roberts
Tel: 072 184 8642
Fax: N/A
Email: laurar@elsenburg.com

APPLICATION TO CONDUCT A CLINICAL STUDY: Rec256-19

Your application to conduct the clinical study refers:

Please find the attached recommendations from the Veterinary Clinical Committee for your attention.

Chairperson: Prof Helen Rees • Vice-Chairperson: Ms Mandisa Hela • Mr Tinyiko Baloyi • Prof Shabir Banoo • Adv Hasina Cassim
Prof Ames Dhai • Prof Craig Household • Dr Edith Madela-Mntla • Dr Ushma Mehta • Dr Mphane Molefe
Dr Thapelo Motshudi • Prof Jeffrey Mphahlele • Mr Itani Mashau • Prof Patric Demana • CEO: Dr Boitumelo Semete-Makokotlela

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PRODUCT NAME: Avian influenza inactivated vaccine
SPECIES: Avian
APPLICANT: Department of Agriculture

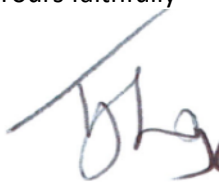
SAHPRA Approval Number: VCT 08/2020

STUDY TITLE: Vaccination of African penguins (Spheniscus demersus) against clade 2.3.4.4 H5 highly pathogenic avian influenza (HPAI).

VCC RECOMMENDATIONS

1. The applicant **has** addressed all the safety and efficacy matters
2. Applicant may commence the study on condition that authorisation is obtained from the Directorate: Animal Health in terms of the Animal Diseases Act no 35 of 1984

Yours faithfully



Dr A T Sigobodhla
DD: Veterinary Medicines Unit
SAHPRA

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SAHPRA
South African Health Products Regulatory Authority

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UNIVERSITY OF PRETORIA
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CSIR Campus
Building 10F
Meiring Naudé Road
Brummeria
Pretoria

Tel (012) 842 7587
pervetS21@sahpra.org.za

Enquiries: Dr A.T Sigobodhla
References: 26/2/2 (VCT/08/2020)
19 May 2020

Department of Agriculture
Elsenburg, Muldersvlei Road
WESTERN CAPE
7607

Att: Dr L. Roberts
Tel: 072 184 8642
Fax: 086 674 6847
Email: laurar@elsenburg.com

PERMISSION FOR USE OF UNREGISTERED PRODUCTS IN TERMS OF THE PROVISIONS OF SECTION 21 OF ACT 101 OF 1965.

Your application refers:
Authorization is hereby granted for the import /purchase of the following unregistered product on condition that:

a. Authorisation is obtained from the Directorate: Animal Health in terms of the Animal Diseases Act no 35 of 1984

Name of product:	Avian influenza inactivated vaccine x 10 ml (5ml of each vaccine)
Species:	Avian
Description of patients:	Penguins
Diagnosis/purpose:	Prevention of avian influenza in birds
Authorisation number:	VCT/08/2020

Kindly note that this permit **allows you a once-off supply** of the imported product for use in the clinical trial. You are reminded to furnish this office with an interim report on the clinical trial.

Yours faithfully

Dr A T Sigobodhla
DD: Veterinary Medicines Unit
SAHPRA

Chairperson: Prof Helen Rees • Vice-Chairperson: Ms Mandisa Hela • Mr Tinyiko Baloyi • Prof Shabir Banoo • Adv Hasina Cassim
Prof Ames Dhai • Prof Craig Household • Dr Edith Madela-Mntla • Dr Ushma Mehta • Dr Mphane Molefe
Dr Thapelo Motshudi • Prof Jeffrey Mphahlele • Mr Itani Mashau • Prof Patric Demana • CEO: Dr Boitumelo Semete-Makokotlela

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Ethics approval for Chapter 6: Planning for disease outbreaks and mass mortality events in South African seabirds

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Faculty of Veterinary Science
Research Ethics Committee

13 January 2023

LETTER OF APPROVAL

Ethics Reference No	**REC063-22**
Protocol Title	**Contingency planning for disease outbreaks in coastal seabird populations**
Principal Investigator	**Dr LC Roberts**
Supervisors	**Prof DA Abernethy**

Dear Dr LC Roberts,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC063-22) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. Note: All FVS animal research applications for ethical clearance will be automatically rerouted to the Animal Ethics committee (AEC) once the applications meet the requirements for FVS ethical clearance. As such, all FVS REC applications for ethical clearance related to human health research will be automatically rerouted to the Health Sciences Research Ethics Committee, and all FVS applications involving a questionnaire will be automatically rerouted to the Humanities Research Ethics Committee. Also take note that, should the study involve questionnaires aimed at UP staff or students, permission must also be obtained from the relevant Dean and the UP Survey Committee. Research may not proceed until all approvals are granted.

We wish you the best with your research.

Yours sincerely

Mrs. MR Watson-Kriek
Chairperson (acting): Research Ethics Committee

Room 6-6, Arnold Theiler Building
University of Pretoria, Faculty of Veterinary Science
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Tel +27 (0)12 529 8390
Email marie.watson-kriek@up.ac.za
www.up.ac.za

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Faculty of Humanities
Fakulteit Geesteswetenskappe
Lefapha la Bomotho

Humanities 100. Since 1919

18 July 2022

Dear Dr LC Roberts

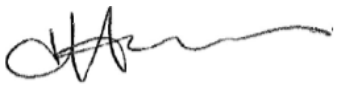
Project Title:	Contingency planning for disease outbreaks in coastal seabird populations
Researcher:	Dr LC Roberts
Supervisor(s):	Prof DA Abernethy
Department:	Production Animal Studies
Reference number:	26217369 (REC063-22)
Degree:	Doctoral

I have pleasure in informing you that the above application was **approved** by the Research Ethics Committee on 30 June 2022. Please note that before research can commence all other approvals must have been received.

Please note that this approval is based on the assumption that the research will be carried out along the lines laid out in the proposal. Should the actual research depart significantly from the proposed research, it will be necessary to apply for a new research approval and ethical clearance.

We wish you success with the project.

Sincerely,



Prof Karen Harris
Chair: Research Ethics Committee
Faculty of Humanities
UNIVERSITY OF PRETORIA
e-mail: tracey.andrew@up.ac.za

Research Ethics Committee Members: Prof KL Harris (Chair); Mr A Bizos; Dr A-M de Beer; Dr A dos Santos; Dr P Gutura; Ms KT Govinder Andrew; Dr E Johnson; Dr D Krige; Prof D Maree; Mr A Mohamed; Dr I Noomé, Dr J Okeke; Dr C Puttergill; Prof D Reyburn; Prof M Soer; Prof E Taljard; Ms D Mokalapa

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