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This document contains the guidelines to manage future outbreaks of avian influenza in sandwich terns.

The document was developed based on information collated during an online bird flu workshop in October 2022 as well as input from experts in virology, epidemiology and bird ecology who attended the second bird flu workshop in Wilhelmshaven in March 2023. It provides advice on several topics such as management options, research and monitoring needs, and safety precautions. The document has been shared with relevant contact persons throughout the Wadden Sea and beyond.

Proposal: WSB is invited to take note of the document.

Sandwich terns on Norderoog, Germany © Bernd Hälterlein/LKN.SH

Management guidelines

Mitigation and data collection strategies for avian influenza in bird colonies in the Wadden Sea

Version: 1.0.2
Authors: Thomas Bregnballe, Kristine Meise, Florian Packmor

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Authors

Thomas Bregnballe, Danish Centre for Wadden Sea Research, Aarhus University

Kristine Meise, Common Wadden Sea Secretariat

Florian Packmor, Lower Saxon Wadden Sea National Park Administration

Editors

Kristine Meise, Common Wadden Sea Secretariat

Cover Photo

Bernd Hälterlein, LKN.SH

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Content

1. This document

This document has been developed to guide site managers (including wardens and rangers), bird counters, researchers and other people that will get in contact with colonially breeding birds in the Wadden Sea. The guide provides advice on several topics such as management options, research and monitoring needs, and not the least, what safety precautions need to be taken. The activities suggested in this document are not meant to be implemented by the general public, given the risk associated with dealing with infected birds and the potential disturbance that could be caused if activities are implemented by large numbers of untrained people.

Further, it needs to be noted that this document is specifically developed for Sandwich terns, which means that some recommendations, e.g. removal of carcasses, may not be a suitable management option for other affected species which are not breeding in dense colonies or are prone to disturbance. Here, separate assessments should be made for each species. However, the guidelines do also provide general advice that will be useful for dealing with outbreaks in other species. Be aware that this guidance document is a living document – it will be updated as and when new information and evidence becomes available. Please check that you are referring to the latest version.

These guidelines were prepared by Thomas Bregnballe (the Danish Center for Wadden Sea Research), Kristine Meise (Common Wadden Sea Secretariat, CWSS & Wadden Sea Flyway Initiative, WSFI) and Florian Packmor (Lower Saxon Wadden Sea National Park, NLPV) (TB and FP are also members of the Trilateral expert group on breeding birds), based on information collated during an online bird flu workshop organised by the CWSS and the NLPV in October 2022 (‘Development and consequences of the recent bird flu outbreak among Sandwich terns in the Wadden Sea and adjacent areas’, see El-Hacen 2022) as well as on input from experts on virology, epidemiology and bird ecology who attended the bird flu workshop held in Wilhelmshaven in March 2023 (‘Identifying management priorities for potential bird flu outbreaks during the next breeding season’). We have also considered safety protocols developed in the United Kingdom and Norway. The document includes some but far from all relevant references to the scientific literature and reports where most of the documentation for given statements and recommendations can be found.

Funding for the development of this document was provided by the Trilateral Wadden Sea Cooperation. The workshops held in October 2022 and in March 2023 were funded by the Landesbetrieb für Küstenschutz, Nationalpark und Meeresschutz in Schleswig-Holstein (LKN.SH) and the Bundesamt für Naturschutz (BfN) with resources provided by the Bundesministerium für Umwelt, Naturschutz, nukleare Sicherheit und Verbraucherschutz (BMUV).

2. Introduction

The high pathogenic avian influenza (HPAI) epidemic in 2021/2022 was the largest observed so far in the EU in terms of number of poultry outbreaks, geographical spread and number of dead wild birds recovered and tested positive (http://hpai.efsa.aus.vet/, Kuiken & Cromie 2022, Pearce-Higgins et al. 2022, Lean et al. 2023). The range of wild bird species affected by HPAI viruses also expanded across Europe, with colonially breeding waterbirds and seabirds of different species being hit by an unprecedented outbreak of high pathogenic avian influenza (HPAI of subtype H5N1) during the breeding season of 2022 (Pearce-Higgins et al. 2022, Boulinier 2023, Lean et al. 2023).

In the Wadden Sea, the Sandwich tern (Thalasseus sandvicensis) together with the Common tern (Sterna hirundo) and the Black-headed gull (Chroicocephalus ridibundus) were the most severely affected species (e.g. Rijks et al. 2022, Pohlmann et al. 2023).

Many discussions were being held in 2022 regarding the suitability of different management measures, but as available data were sparse, decisions were taken on an individual basis. With many species now already returning to their breeding areas in the Wadden Sea, it was essential to draw conclusions from actions that were previously taken, prepare advise on how to deal more effectively with possible outbreaks during upcoming seasons, but also determine how to assess the impacts the HPAI outbreak had on the most affected bird populations.

Consequently, the Common Wadden Sea Secretariat (CWSS), as the coordinating unit for the Trilateral expert groups on breeding and migratory birds and the Wadden Sea Flyway Initiative, decided to develop this guidance document, which will provide important information on precautionary measures and necessary data collection to better understand the dynamics of HPAI outbreaks as well as possible management options for Sandwich tern colonies, the most badly affected species in the Wadden Sea.

Outbreaks of avian influenza, also among Sandwich terns, have recently been reported from Senegal and The Gambia. In addition, Sandwich terns breed in close contact with Black-headed gulls, and in the early spring of 2023, gulls (in particular Black-headed gulls, https://eurlaidata.izsvenezie.it/) have been among the most affected wild bird species in Europe. It is therefore not unlikely that breeding colonies of Sandwich terns in the Wadden Sea will be exposed to HPAI in the coming season.

3. Transmission routes and immunity

Spread of disease via movements of birds

Avian influenza is thought to be primarily spread by movements of infected birds. Migratory birds, especially waterbirds, can carry the virus along their migration routes. Certain species, such as dabbling ducks (e.g. Mallards *Anas platyrhynchos*), do not show signs of a clinical infection but may shed the virus during infections, thus contributing to its spread (e.g. see Ahrens et al. 2022). Furthermore, it is common that colonial birds move between colonies also within the breeding season (Spaans et al. 2021) and individual birds may thereby disperse avian influenza from one colony to another.

Bird-to-bird contact

Avian influenza can spread through direct contact with live conspecifics (e.g. in high density breeding colonies) as well as via direct interactions with other species (e.g. through kleptoparasitism or aggressive behaviour) (Camphuysen & Gear 2022, Adam et al 2023, Boulinier 2023). The virus can also spread via contaminated body fluids and through interactions with sick or dead conspecifics (e.g. because of scavenging behaviour of large gulls) (Camphuysen & Gear 2022, Adam et al 2023, Pohlmann et al. 2023). It is uncertain at present whether transmission via direct bird-to-bird contact (i.e. via physical interactions with other live, sick and dead birds) is the main driver of infection of wild birds (Camphuysen & Gear 2022, Adam et al. 2023), but experts agree that the persistent presence of dead birds which have succumbed to the disease constitutes a threat to live birds in the vicinity for several months (Yamamoto et al. 2017).

Spread of disease through uptake from the environment

Avian influenza can also spread indirectly by ingestion of infectious material such as contaminated freshwater, contact with faeces from infected birds or other contaminated objects and surfaces (Camphuysen & Gear 2022, Pohlmann et al. 2022, Adam et al. 2023). For example, influenza A viruses are known to remain infectious for several months in freshwater at low temperatures, suggesting that waterborne transmission can be an important driver of infection in aquatic birds (Domanska-Blicharz et al. 2010, Ahrens et al. 2022, Ramey et al. 2022). However, as mentioned, it remains uncertain how much indirect transmission due to environmental contamination contributes to transmission between wild birds or whether the main transmission occurs via direct bird-to-bird contact (Adam et al 2023).

Heterospecific bird species which are carriers of the disease are likely to play an additional role in transmission of avian influenza, also to Sandwich terns, through contamination of the environment (e.g. via infected faecal material and fresh carcasses). A freshly dead bird lying in a pool of freshwater can contaminate the water where terns and other species preen and

bathe (these are sometimes called “death pools”) (Pohlmann et al. on dead wild birds 2022). Wild birds can also transport live virus in their plumage.

Spread of disease by human activities

Humans can contribute to the spread of avian influenza through direct contact, for example by handling infected birds and not properly disinfecting afterwards, or through indirect contact (e.g. through transfer of faeces and feathers). It is not clear for how long H5N1 virus can survive in organic matter such as soil and nesting material, but it might be able to survive for several weeks if it is not exposed to UV light, high temperatures or very dry conditions (Domanska-Blicharz et al. 2010, Nazier et al. 2010, Keeler et al. 2014).

Development of immunity

New variants of avian influenza constantly develop and while some of them may have low impact on certain species of waterbirds, others can cause severe illness and death soon after infection. Little is currently known about the ability of Sandwich terns to develop immunity against the various forms of HPAI and the duration of immune protection. While there is hope that at least some (and potentially many) adults have developed immunity or will develop immunity, chicks are likely unable to develop immunity for HPAI during the first weeks after hatching, making them highly susceptible to HPAI. We assume that this explains why in many of the Sandwich tern colonies studied in 2022 large numbers of chicks (and in some cases almost all chicks) died before fledging, even in colonies with relatively low adult mortality. The generally highly efficient transmission of maternal antibodies through egg yolk in birds (Trampel et al. 2006, Koethe et al. 2020), gives hope for better immunity in chicks for the upcoming influenza season.

4. Management options: precautions to reduce the risk of spreading highly pathogenic avian influenza

Experts in ornithology, virology, and epidemiology who attended the workshop held in March 2023 evaluated the chance of a Sandwich tern breeding colony getting infected by HPAI in the Wadden Sea, considering the current knowledge on the H5N1 HPAI virus and Sandwich tern behaviour. The experts identified possible entry and exposure pathways and based on the likelihood and frequency of occurrence of an infection through certain pathways, they classified pathways into ordinal-scaled risk categories (One Health High-Level Expert Panel 2022). For pathways evaluated as ‘moderate’ to ‘very high’ risk, a set of

mitigation measures was developed with the objective to minimize the risks of entry and risks of exposure.

Entry pathways

There are still many unknowns and even contrasting scientific evidence regarding possible entry pathways. It is likely that multiple pathways contribute, making it difficult to decide on adequate mitigation measures that cover all possible entry routes of the virus. Regardless, experts suggested a number of precautionary measures that should minimize the likelihood of a HPAI virus introduction.

The group of experts recommend implementing the following measures ***to prevent the entry of the virus into the colonies:***

1. **Prevention.** All personnel scheduled to enter a colony during the breeding season should receive training on the proper use of protective gear, disinfectants, and the proper disposal of used (and potentially contaminated) gear and should follow advice given under “6. Precautions and safety measures”.
2. **Breeding sites.** Active, previously used and potential breeding sites of terns and gulls should be identified, and fresh carcasses should be removed from active and potential breeding sites until April 10 (see Box A).
3. **Spreading of risks.** Sandwich terns may be actively attracted to alternative breeding sites that appear to provide a good chance of a successful breeding season (using decoys and sound lures, see section 4.6).
4. **Early detection.** The situation in the breeding colonies should be constantly monitored once Sandwich terns start returning to the Wadden Sea by different means: drones, live cameras, and physical visits. The focus is on Sandwich terns and Black-headed gulls who share colonies, but unusual events in other potentially vulnerable species should be considered (see section 4.1).
5. **Carcass removal.** Once dead or sick birds are detected in breeding colonies, at least five carcasses should be tested for avian influenza and all dead individuals should be removed or buried deeply. All equipment which is needed for this task (including personal gear and monitoring equipment) needs to be thoroughly cleaned and disinfected when leaving the site and again when entering a breeding colony to minimize the risk of spread via human activity at the breeding site, and when moving from the island to the mainland and to other breeding sites (see section 6).

Dead Sandwich terns documented on an island in the Schleswig Holstein Wadden Sea. © Bernd Hälterlein/LKN.SH

Exposure pathways

Experts agreed with high certainty that the interactions between conspecifics, including interactions with sick and dead birds, play a key role in the transmission of the virus. Interactions with individuals from other species frequently found in Sandwich tern breeding colonies were also assigned a high risk.

The following measures were identified and discussed ***to further reduce transmission of HPAI within the colonies (exposure):***

1) **Carcass disposal.** Proper and safe disposal or deep burial of potentially large numbers of carcasses (adults as well as chicks) needs to be ensured and organized in collaboration with the respective veterinary authorities (see Appendix 2).
2) **Removal frequency.** Carcasses of Sandwich terns and other species should be removed regularly (ideally every day, or as often as possible), especially at the early stages of an outbreak. The area where the carcass was removed could be treated with salt to reduce the viral load. Mortality rates should be monitored to determine when measures can/should be stopped (see section 4.2) to minimize disturbance.
3) **Sick bird surveillance.** The location of sick birds with clear clinical signs of HPAI should be noted and controlled the following day to remove potential carcasses (and acquire potential information regarding the course of the disease) (see section 4.3).
4) **Communication.** Information on any confirmed and potential cases of HPAI in proximity of the Sandwich tern colonies should immediately be shared with site

managers as well as the wider Sandwich tern conservation community (see section 7 on planned communication channels).

5) **Compartmentation.** At least in some colonies, compartments (e.g. low fences) should be set up to minimize the chicks' range of movement within the colony for assessing their impact on the spread of the disease (see section 4.4).

6) **Public feeding.** During active virus circulation (evidence of virus in the region within 3-4 weeks), public feeding of waterbirds should be banned, and frequently used feeding sites should be patrolled by rangers and law enforcement. While Sandwich terns do not use public feeding sites, species like Black-headed gulls and Herring gulls do, and these species could contribute to the spread of the virus in the colonies.

7) **Environmental risks.** Freshwater ponds in the vicinity of colonies should be monitored for their potential role in the spread of the virus. Removal of carcasses from the ponds and the edge of ponds is strongly recommended (see section 4.5).

We recommend that site managers keep a record of any management interventions, e.g. whether carcasses have been removed or left in-situ. This will help gather evidence with respect to the impacts of interventions and improve future guidance. Remember to follow the advice given under "6. Precautions and safety measures".

4.1 Detection of HPAI

The presence of a larger number of dead (and/or appearing sick/ill) individuals of a species (e.g. >4 sick adult birds/fresh carcasses detected within five days) in a colony or its surroundings makes an HPAI infection likely. Be aware that there can be lots of dead chicks in a colony for other reasons than illness, e.g. due to bad weather.

Clinical signs

Characteristics of the apparently sick individuals can also be used as indications that birds have become ill from HPAI. The clinical signs of avian influenza in birds include:

- closed and excessively watery eyes
- lethargy, recumbency and unresponsiveness
- incoordination and loss of balance
- head and body tremoring
- drooping of the wings or dragging of legs
- twisting of the head and neck

While the clinical signs outlined above can indicate avian influenza, the presence of avian influenza virus can only be confirmed through laboratory tests by the respective local authorities.

Early detection of disease in colonies

For some colonies, there might be alternative ways to monitor them than regular physical visits to the islets and breeding sites. Thus, it may be advantageous to use a drone to determine if/when birds start to die or for how long they are continuing to die. If a drone is being used, we recommend gathering information by both filming and taking photographs. This is best done from a low height (e.g. down to 15-20 m above the colony) to reduce the risk that dead individuals in the vegetation are overlooked; incubating Sandwich terns and Black-headed Gulls would normally not leave their nest despite a low flying drone. There may be circumstances, however, when birds respond to the drone. It is very important to consider and react to the birds' response and reduce the disturbance as much as possible. Further, any use of drones has to follow the local regulations and guidelines for the application of UAVs in wildlife monitoring. It might also be possible to install and use wildlife cameras or video surveillance cameras in the colony that continuously transmit photos or videos to a mobile phone or a computer. However, these devices cover only restricted sections of the entire breeding area. Because patterns of mortality are unlikely to be evenly distributed over the breeding area and because sick individuals may end up dying outside the colony, there is a risk of missing the onset of an infection by the use of these devices. Vegetation growth may make it more difficult to successfully use remote sensing to detect HPAI infections later in the breeding season, but the use of infrared tools could potentially aid in the detection of bodies in the vegetation.

Confirmation from analyses of freshly dead individuals

Sampling for necropsies. Select five (or more) freshly dead individuals per species (give priority to ringed individuals) from each colony to get them analysed for presence of HPAI and genetic analysis. In case of larger outbreaks, it may be beneficial to increase the number of carcasses for genetic investigation to better understand potential routes of transmission. For further information see section 5.2.

Swab samples. Oropharyngeal and cloacal swab samples should be taken from at least five of the fresh carcasses and carcasses should be sent for detailed analysis to the regional investigation center. Such samples should also be taken from the individuals that will be delivered for necropsies. In an ongoing HPAI event in the colony, further samples should be taken after 2-3 weeks. At sites where it is relevant to quickly get an indication whether or not the sick and dead birds have HPAI it can be considered to conduct rapid tests for influenza virus (see section 6.1).

4.2 Removal of dead birds

Throughout the winter the virus has been detected in several waterfowl and coastal bird species, including geese, ducks, and gulls. Carcasses have been found on beaches and at potential breeding sites of Sandwich terns. Fresh carcasses are likely to serve as a source for

infections as they can contain an extremely high viral load that may infect birds and other animals (Yamamoto et al. 2017).

Collecting bird carcasses protects other individuals from coming into close contact with the virus. This is particularly true for species scavenging on the carcasses (e.g. gulls, corvids, but also mammals like foxes, otters, or seals scavenging on dead wild birds) (Camphuysen & Gear 2022, Aguero et al. 2023, Bordes et al. 2023, Boulinier 2023). The current experience from Sandwich terns suggests that removing carcasses at an early stage of an outbreak can reduce the mortality of both adults and chicks (Rijks et al. 2022, unpublished data). The fresher the carcass, the more virus it will contain. It is therefore recommended to remove carcasses as soon as possible after death. The area where the carcass was removed could be treated with salt to reduce the viral load in the soil underneath carcasses.

In some colonies and phases of the breeding cycle, searches for and removal of dead birds can increase aggression between the birds and cause abandonment of nests and dispersal, which potentially may add to the risk of spreading the disease between colonies. Disturbance later in the breeding season can also impact breeding success. Nonetheless, given the impact of the disease on the survival of adult birds, we recommend regular visits (always minimising disturbance) to remove dead birds, despite the disturbance caused by such activity. Disturbance might be minimised by remote surveillance of mortality via the use of wildlife cameras, video surveillance cameras or drones (see also 'Early detection of disease' under section 4.1). Such tools may also help to determine whether or not a visit can be postponed. During ongoing strong outbreaks with large numbers of infected/dead birds, it should be decided at the site level if the removal of carcasses should be stopped to avoid further disturbance. Here, it is important to consider, among other factors, the risk of additional species being exposed (including avian and mammalian scavengers).

Remember that those who collect the carcasses will need to have proper training and protective equipment and follow their organization's relevant health and safety policies and risk assessments (see "6. Precautions and safety measures").

Box A. Removal of dead birds

We recommend

- Removal of dead birds from breeding sites before the onset of the nesting season, e.g. before April 10 in Sandwich tern colonies.
- Active surveillance (direct observations or remote surveillance) of colonies from the onset of the breeding season to determine if and when birds start to die.
- Removal of dead birds from within or anywhere near the colony, as they appear.
- Removal of dead birds (including chicks) to be continued throughout the breeding season. If and when the removal of carcasses should be stopped during ongoing strong outbreaks with large numbers of infected birds should be decided on an individual site level.
- Use of protective equipment as outlined by the relevant organisations.
- Follow the guidance on how bird carcasses suspected of having HPAI should be disposed of (see also Appendix 2).

Handling and disposal of dead birds

Dead or sick wild birds should not be touched without the appropriate protective equipment (see “6. Precautions and safety measures”).

Each dead bird should be sampled in a leakproof plastic bag which must be tied. Insert this bag in a secure container or an extra plastic bag if no secure container is available. The birds should be disposed of as an animal by-product. On remote islets it is recommended to bury the carcasses without plastic bags at a sufficient depth, to prevent them being washed out during flooding.

4.3 Monitoring and managing of sick birds

Given the very high probability of death in individual Sandwich terns showing symptoms of avian influenza, euthanizing very sick individuals may help to reduce the spread of the virus. However, there are legal obligations to consider. It is, for example, not allowed in the Netherlands, Germany and Denmark to take the life of a diseased wild bird (unless the bird is taken into care and the person is equipped with a special permission), so please seek legal advice before undertaking such actions. Any admission to and treatment of such birds in sanctuaries is expected to be contra-productive and could promote virus spread. In any case, the location of sick birds in the colony should be noted, so that the area can be surveyed for dead birds in the following days.

4.4 Compartmentation to protect chicks

As mentioned at the beginning of this document, chicks are likely to be particularly susceptible to dying from infections with HPAI. This was evident in most of the Sandwich tern colonies followed during the 2022 outbreak. While direct interactions between adults and chicks, through feeding, will contribute to the transmission of the virus, the role of interactions between (potentially infected) chicks is unknown. Dividing colonies into sections by putting up physical barriers to keep infected chicks from aggregating in large groups, may reduce the transmission of avian influenza among chicks from different parts of the colony.

4.5 Reducing transmission through freshwater ponds

In colonies where the birds have access to ponds of standing freshwater, they may get infected by contact with the water, especially if dead birds have been lying in it (Ahrens et al. 2023). Covering/closing small ponds with a high potential to transmit the virus (environmental surveillance is needed) may be beneficial (Ramey et al. 2022), but depends on the availability of alternative waterbodies for bathing and drinking nearby. At some sites, it might be possible to turn freshwater into saltwater, which would reduce the environmental sojourn time of the virus. However, such measures should only be taken in collaboration with

the landowners and the responsible authorities, and after an assessment of the potential impact on the environment and local biodiversity.

4.6 Improving the availability of alternative breeding sites

Long-term it will benefit a species like the Sandwich tern to have access to a larger number of attractive breeding sites. Preliminary analyses suggest that overall fewer individuals will die from HPAI if the breeding birds are dispersed over more sites with fewer breeders being present at every single site. In 2022 it was recorded that the risk of severe infection with HPAI was positively related to colony size, i.e. large colonies were at a higher risk of receiving individuals that would carry and spread HPAI (large colonies attract more birds than smaller colonies). However, Sandwich terns do not only select new breeding sites based on where conspecifics have settled or the presence of Black-headed gulls, which provide protection against common predators, but also to a large extent on the likelihood of sufficient food availability (fish of the right species and sizes) throughout the breeding season.

5. Research and relevant information to collect

Research on how avian influenza viruses are emerging and spreading in wild populations is urgently needed. We still know very little about many facets of the disease, so it is important to collect baseline data to be able to understand the impacts of HPAI on wild bird populations from a conservation perspective and to enable us to better manage outbreaks and limit mortality in the future (Camphuysen & Gear 2022, Rijks et al. 2022). Comparisons between colonies with different scales of outbreaks of HPAI can help to shed some light on factors that affect transmission routes or susceptibility to the virus. Therefore, it is essential that researchers collect information on the same variables when visiting colonies that have experienced or are experiencing an HPAI outbreak, which can later be analysed. Here, we provide a set of variables which have been determined as important by researchers involved in the monitoring of last year's HPAI outbreak among Sandwich terns. However, establishing a research protocol in advance can be difficult, given the highly variable nature of infections we have witnessed in previous years, even between colonies of the same species. Opportunistic observations should therefore always be collected and shared as they emerge. Above all, however, we need to gather data that will help us to estimate the impacts of avian influenza on wild bird populations.

5.1 Live birds – active and sero-surveillance

Sampling from live birds can help to understand why some bird species seem more susceptible to certain HPAIV strains than others. Is this an innate immunity or has the susceptibility to clinical or fatal disease decreased over time through exposure to different strains of LPAI/HPAI viruses? What is the survival rate of birds with or without immunity?

Swabs

Take combined pharyngeal swabs and cloacal swabs of birds being handled (e.g. for tagging and ringing). See Appendix 2 for country specific guidance on how to take swabs, how to store them, and deliver them to the relevant laboratories.

Blood samples

If possible, serum of adults or eggs for sero-surveillance should be sampled. This will provide insights into the proportion of natural antibody prevalence (against LPAI and HPAI) which would give an indication of how susceptible the population is towards HPAI infections. This requires a) permissions from authorities, b) the correct equipment, c) agreements with laboratories that can process the samples, and d) that appropriate procedures are followed with respect to storage and delivery to relevant laboratories (see Appendix 2).

5.2 Sick or dead birds

Once you have prepared yourself to handle sick or dead birds (see section 4.2 and section 6), we recommend the following data collection protocol:

- Count all dead birds of each species present. See Appendix 2 for how to report on numbers of dead birds found.
- Distinguish between adults and chicks (and sexes if possible) when counting dead Sandwich terns. Age the chicks by measuring the total length from the back of the head to the tip of the bill (known as total head length). And/or give an estimate of the age of the chicks based on the following pictures (taken by Ulrich Bolm-Audorff, Verein Jordsand):

Newly hatched (0-3 days) | 4-9 days | 10-20 days | 21-30 days

- Count the number of sick birds of all species present and take notes about their location in relation to the colony, the shore, and water ponds.
- For each dead individual denote whether it was freshly dead, dead for more than a week, longer dead, or skeletonized.

- Check all dead birds for rings. Collect the rings and report the information to the ringing centre in your own country/region. Ring recoveries provide extremely valuable information about wild bird populations and their movements, as well as information on mortality.
- Select five (or more) freshly dead individuals from each colony to determine the presence of HPAI and conduct a genetic analysis. Store them in two plastic bags and add labels to the bags with information on the species and the date of collection (see section 4.2). Freshly dead individuals collected for laboratory analysis should be kept cool (e.g. 5-8° C) for up to 3-4 days. Only in exceptional cases should birds be frozen directly after having been collected if they are not going to be analysed within 4 days from collection. Fresh carcasses can be frozen directly after having been collected. Freezing the birds reduces the possibilities for histological and bacteriological examinations but has very limited impact on PCR analyses. The system for delivery of freshly dead individuals for HPAI testing varies among the countries (see Appendix 2).

5.3 Breeding colonies

We recommend that the following type of information is collected during visits to the breeding colonies:

- The number of breeding pairs. Consider whether it might be an advantage to use a drone for counting.
- The stage of the breeding cycle at the time of visit, e.g. eggs, hatching and age of chicks.
- The presence of other bird species within or in the vicinity of the breeding colony (including number and breeding status).
- Take note of the presence of ponds and environmental structures within and in the surroundings of the colony.
- Take photos of the colonies at each visit. This is helpful for later descriptions of the colony type with respect to the amount of vegetation among the nests.

5.4 Sampling of the environment

We urgently need a better understanding of the prevalence and potency of the disease in the environment of colonies. If collaborations with a laboratory can be developed, we recommend collecting samples a) of surface water of small freshwater pools and ponds with brackish water adjacent to breeding sites, and b) of faecal matter of different species as well as c) sediment samples from shallow ponds. Experimental designs are required to aid the interpretation of environmental data and national laboratories are advised to get in contact to coordinate the experimental design. Further guidance will be shared as it becomes available.

5.5 Monitoring reproductive success, movements, and survival

To gain knowledge about the impact of avian influenza on the populations of seabirds and colonial waterbirds, it is of interest to monitor the production of young and gather information that can be used for estimating survival.

Bird ringing is a very useful method allowing us to identify individual birds. Ringing birds with metal rings and colour rings engraved with letters and numbers can help to a) document dispersal and movements of individuals, b) estimate breeding success and c) create the possibility for collecting data that will later allow us to estimate mortality rates. Please report all rings observed or found in colonies and beyond.

Always ensure that equipment used for ringing purposes is thoroughly cleaned and disinfected (see section 6.1). For specific information about precautions to take relating to bird ringing activities in areas with HPAI risks, please see guidelines from your own ringing centre or institution.

Breeding success

Recommended methods for monitoring breeding success of Sandwich terns are described in Koffijberg et al. (2011). Besides the methods described in this manual, it is also possible to estimate the survival rate of chicks from hatching to fledging by ringing some of chicks when they are small and returning to the breeding site just before the chicks start to fledge to record the proportion of chicks that carry a ring.

Movements

It is highly relevant to collect information on how individuals disperse and move between breeding sites. This can be studied by systematic reading of rings at or near the colonies and by use of GPS-tagging of adults. The reading of rings becomes far easier if the chicks and adults have been ringed with colour-rings or special metal-rings designed for reading from a distance.

Survival

Information from recoveries of individuals ringed as chicks or adults can be used for estimating survival in the population. The strength of such analysis can be improved if systematic attempts are made to read the rings at or near the breeding sites and/or at staging sites.

6. HPAI precautions and safety measures

There are good reasons to take precautions when entering or working inside breeding colonies. One reason is to limit the risk of spreading the virus within the colony, to other colonies and to sites located away from the colonies. In addition, it minimizes the risk of human infections. Although transfer of HPAI to humans is extremely rare, it can happen, and some infections have been fatal. This highlights the need to reduce any risk of exposure for people directly working with infected, sick, or dead birds or contaminated animal/environmental products.

6.1 Precautions to be taken

Guidance on precautionary measures to be taken when working with wild birds in areas where the birds are potentially affected by HPAI varies between countries, authorities and employers. It is likely that your own organisation has Health and Safety policies and risk assessments. If so, please refer to these in the first instance, but also familiarize yourself with other protocols.

All people that get in contact with potentially infected wild birds and contaminated substrates should receive an adequate training session on health and safety measures before starting field work.

Our general advice on measures to take is:

- Always use Personal Protective Equipment (PPE) as described in Box B.
- Clean footwear before and after each colony visits (also when moving between subcolonies) using disinfectant. Footwear needs to be clean of substrate before they are disinfected. Apply disinfectant solution until it runs off or dip in disinfectant. Be aware of the potential biohazard activity of the disinfectant in the environment.
- Clean all equipment with disinfectant. Spray the equipment with disinfectant solution until it runs off.
- Keep dead birds in secure containers (see section 4.3).
- Dispose of dead birds according to the regulations given by your country's or region's authorities (see section 4.3 and Appendix 2).

Cleaning and disinfecting contaminated footwear, clothing and equipment significantly reduces the risk of transferring the H5N1 virus. Use sealed containers to transport soiled clothes, equipment and sanitization materials. Dispose of potentially contaminated materials in domestic or commercial waste. Avoid using fieldwork clothes for other purposes. Any clothing that has been in contact with dead birds should immediately be washed after return from the field.

It can be relevant for researchers, managers, or ringers to get an indication on site if the birds being handled or found freshly dead have HPAI. In that case, it is possible to conduct rapid tests for influenza virus, taking pharyngeal swabs and cloacal swabs. The testers should be trained in handling rapid tests beforehand, furthermore, appropriate equipment is needed on site for safe disposal of the tests. A positive test is a good indication of an infection with influenza type A virus, which need to be confirmed by a standard laboratory analysis. A negative result does not rule out a virus infection as rapid tests are not as sensitive as PCR. Therefore, rapid tests cannot and must not replace the lab diagnosis of freshly dead individuals.

Box B. Your own personal hygiene

Above all else, be careful about your own personal hygiene:

- We recommend that you familiarize yourself with training videos that carefully explain how to dispose of infectious protective gear (see video in Appendix 1).
- Always wear protective clothing preferably Personal Protective Equipment (PPE) – disposable overalls, rubber/polyurethane boots (or disposable shoe covers), disposable FFP2 masks (or full-face respirator), safety goggles, disposable nitrile/vinyl/heavy duty rubber (not latex) gloves.
- Avoid handling dead birds, or life birds which may be infected, their droppings, or any water nearby, with your bare hands. Consider that some species do not show symptoms. If handling is required, e.g. to read a ring, disposable nitrile gloves should be worn at all times; if these are not available, an inverted plastic bag can be used.
- Wash hands thoroughly with disinfectant soap and or use anti-viral hand-wash after handling sick, dead or even potentially infected animals or coming into contact with potentially contaminated substrate. Make sure to use a recommended virucide.
- Avoid bringing your hands close to your face until your hands are clean. Do not drink, eat or smoke after handling birds/contaminated substrate if you have not yet properly disinfected yourself.

6.2 What to do in case of symptoms of illness

Transmission of avian influenza to people is a rare event but cannot be excluded when exposed without PPE to infected sick or dead birds. If you have been exposed to likely infected birds or contaminated environment, it is important that you monitor yourself for any symptoms that might occur in the period of 10-14 days after the exposure. Be aware of signs of flu-like symptoms during the first 10-14 days following visits to or work inside breeding colonies where birds have been infected with avian influenza. The symptoms include conjunctivitis, fever with a temperature ≥38°C, cough or shortness of breath requiring hospitalization; but also other symptoms such as gastroenteritis diarrhoea, vomiting, or abdominal pain.

Seek medical advice immediately should you display symptoms of any illness that may be related to ringing or nest recording, inform your doctor or healthcare provider via telephone that you may have been exposed to HPAIV or other potential avian zoonoses and ask them to perform a test for HPAIV and avoid entering health care facilities when having symptoms.

It is recommended that people conducting field work inside breeding colonies have an active vaccination against seasonal human influenza (Gray & Baker 2007). Seasonal influenza vaccines do not protect against avian influenza viruses (Oshansky et al. 2014) but may reduce the risk of a co-infection with a seasonal and avian influenza virus that could lead to the emergence of a reassorted influenza virus. The reassortment process is less likely to occur in vaccinated persons. Antiviral pre- or post-exposure prophylaxis might be available and recommended by the local health authorities and based on a local risk assessment.

7. Tasks for the near future

There is a need to develop an early warning system to inform all relevant personnel about active outbreaks of HPAI in neighbouring or even distant colonies that require their immediate attention.

An internet platform shall be established specifically for Sandwich terns in Europe. One of the ideas is to provide news on this platform and generate a mailing list so that interested people/institutions are notified when new information becomes available.

7.1 Access to guidance

Easy access to guidance on how to clean and dispose of potentially infectious materials (clothes, carcasses, equipment) is urgently needed. Such guidance could be in the form of training videos (for an example, see “How to remove gloves without contaminating hands” in Appendix 1) or infographics.

7.2 Research questions to be studied

It would be relevant to conduct studies or experiments that could provide further knowledge about the transmission of the virus and the effects of infections. Suggested research priorities include:

- Transfer of virus. How do wild birds become infected? When do infected birds become infectious? It has been observed in several colonies that healthy birds pick at sick birds that show abnormal behaviour or even interact with dead birds. Since picking at sick/dead birds can be a way of virus-transfer, it is relevant to gain more knowledge on this. More information on the course of infection will further allow researchers to evaluate the risk of infected birds transmitting the virus between colonies – e.g. how far can they travel when already infected?
- Development of immunity. How large a proportion of the breeding birds have antibodies in the blood that should protect the birds from the severe effects of

infections with HPAI? Do birds develop an immunity that lasts between breeding seasons upon an infection? Is an immunity passed down to chicks via the egg yolk?

- Mortality rates. Better estimates of mortality rates due to HPAI infections and immunity. Coordinate collation of data on numbers of individuals found dead in total and with confirmed HPAI infections.

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Appendix I: Relevant links

Information about HPAI in wild birds

The EFSA report provides an updated overview of the ongoing avian influenza epidemic at European and global level: https://doi.org/10.2903/j.efsa.2023.7917

Reports like the following will be posted bimonthly, the next will be published in May 2023: Avian influenza overview December 2022 – March 2023 (europa.eu)

The avian flu data portal (https://eurlaidata.izsvenezie.it/) is an interactive dashboard to visualize all the HPAI H5 outbreaks reported in wild and domestic birds in Europe using different methods (charts, maps, table).

Concerning wild species of waterbirds a new prototype to show recent outbreaks of HPAI is now available at Early Warning System for HPAI outbreaks in wild birds (EFSA's Bird Flu Radar). The home page includes a short YouTube video that explains how the Early Warning System works.

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1100864/mitigation-strategy-avian-influenza-wild-birds-england-wales.pdf

Avian Influenza in Wild Birds - YouTube

Wild Birds and Avian Influenza - An introduction to applied field research (fao.org)

Health and Safety guidance

Inspiration can be found here:

https://www.hse.gov.uk/ppe/index.htm?utm_source=hse.gov.uk&utm_medium=referral&utm_campaign=guidance-push&utm_term=ppe&utm_content=home-page-popular

https://www.fli.de/en/news/short-messages/short-message/highly-pathogenic-avian-influenza-hpai-risk-assessment-of-the-fli/

https://www.fli.de/de/aktuelles/tierseuchengeschehen/aviaere-influenza-ai-gefluegelpest/

How to remove gloves without contaminating hands

A video can be found here:

https://www.hse.gov.uk/skin/videos/gloves/index.htm

Disinfectants

Details on disinfectants and concentrations required can be found here:
http://disinfectants.defra.gov.uk/DisinfectantsExternal/Default.aspx?Module=ApprovalsList_SI

https://www.gov.uk/guidance/defra-approved-disinfectant-when-and-how-to-use-it

In German:

https://desinfektions-rl.fli.de/de/home

DVG | Desinfektion in der Veterinärmedizin | Ausschuss der Deutschen Veterinärmedizinischen Gesellschaft e.V.: Desinfektionsmittel Tierhaltung (desinfektion-dvg.de)

In case of suspicion of humans infected with avian influenza

In Germany, the RKI has the following guideline for suspicion of humans infected with AI:

RKI - Zoonotische Influenza - Empfehlungen des Robert Koch-Instituts für die Meldung und das Management von Personen mit Verdacht auf aviäre Influenza (Influenzavirus A/H5N1)

Appendix II: Country-specific guidance

This annex is intended to give country specific guidance on how to a) dispose of dead birds, b) deliver dead birds for HPAI testing, c) report on numbers of dead birds found, d) to take, store and deliver swabs. At time of finalizing this document, we did not have information to guide people on all aspects for all areas in the Wadden Sea. We only list areas for which we have information to guide people.

Disposal of dead birds

The Netherlands:

Landowners and site managers are the appropriate party to determine whether and how dead wild birds are removed from the areas they own or manage. For more details please consult the national guidlines

Leidraad omgang met wilde vogels met vogelgriep (overheid.nl)

Handleiding voor het opruimen van dood gevonden wilde (water)vogels | Voorschrift | NVWA

Niedersachsen:

Dead birds should only be collected by trained persons. Please inform Wadden Sea National Park Administration in Wilhelmshaven (https://www.nationalpark-wattenmeer.de/service/kontakt/) or the district office ("Ordnungsämter") and/or the local veterinary office ("Veterinärämter" "; https://www.ml.niedersachsen.de/download/4040/Kommunale_Veterinaerbehoerden_des_Landes_Niedersachsen.pdf).

Schleswig-Holstein:

Dead birds should only be collected by trained persons. Please inform Wadden Sea National Park Administration in Tönning (https://www.nationalpark-wattenmeer.de/service/kontakt/) or the district office ("Ordnungsämter") in other areas.

Denmark:

Dead birds should only be collected by professionals. The dead birds should be delivered to DAKA or in leakproof and sealed plastic bags in normal containers.

Reporting of dead birds for HPAI testing:

The Netherlands:

The DWHC investigates birds with avian flu. Dead wild birds can be reported to DWHC. The responsibility for cleaning up does not lie with DWHC and they do not collect all the reported birds for research.

Meldpagina | Dutch Wildlife Health Centre (DWHC)

Niedersachsen:

Please inform the local authorities (Wadden Sea National Park Administration in Wilhelmshaven (https://www.nationalpark-wattenmeer.de/service/kontakt/) or the district office ("Ordnungsämter") and/or the local veterinary office ("Veterinärämter"; https://www.ml.niedersachsen.de/download/4040/Kommunale_Veterinaerbehoerden_des_Landes_Niedersachsen.pdf).

Schleswig-Holstein:

Please inform the local authorities (Wadden Sea National Park Administration in Tönning (https://www.nationalpark-wattenmeer.de/service/kontakt/) or the district office ("Ordnungsämter") in other areas). In addition, carcasses for necropsy should be announced at Landeslabor Schleswig-Holstein, +49 4321 904-648; tierseuchen@lsh.landsh.de

Denmark:

Install and use the app “FugleinfluenzaTip”. See alsohttps://www.foedevarestyrelsen.dk/Selvbetjening/Guides/Sider/Fund-af-doede-eller-syge-vilde-fugle-i-naturen.aspx

Reporting numbers of dead birds

The Netherlands:

Report dead birds @ Meldpagina | Dutch Wildlife Health Centre (DWHC) or Dode vogel melden | Sovon

Niedersachsen:

Please inform the local authorities (Wadden Sea National Park Administration in Wilhelmshaven (https://www.nationalpark-wattenmeer.de/service/kontakt/)

Schleswig-Holstein:

Please inform the local authorities (Wadden Sea National Park Administration in Tönning (https://www.nationalpark-wattenmeer.de/service/kontakt/) or the Landesamt für Umwelt, Flintbek (https://www.schleswig-holstein.de/DE/landesregierung/ministerien-behoerden/LFU/LFU_node.html)

Denmark:

Send an e-mail to tb@ecos.au.dk. It is the plan to extend the app “FugleinfluenzaTip” so you can report numbers found dead via this app.

Swabs and their delivery

The Netherlands:

Contact DWHC @ Meldpagina | Dutch Wildlife Health Centre (DWHC)

Handleiding voor het opruimen van dood gevonden wilde (water)vogels | Voorschrift | NVWA

Niedersachsen:

Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES).

Contact: Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Postfach 9262, 26140 Oldenburg, +49 441 570 26-0, poststelle@laves.niedersachsen.de

Schleswig-Holstein:

Landeslabor Schleswig-Holstein, Neumünster.

Take and store swabs: Swabs from oropharynx incl. choane and cloacal swab or faeces (combined swab possible); use dry swabs or swabs with transport medium for virological examinations; refrigerated storage and transport within 48 hours. Swabs with medium preserving RNA do not need special transport conditions (expensive, comparative test in preparation);

Transport: Complies with packing instruction P650: primary receptable (swab cover), secondary package (liquid-tight, adsorbing gauze); outer packing (cardboard); cooled conditions;

Contact: Landeslabor Schleswig-Holstein, Max-Eyth-Str. 5, 24537 Neumuenster, +49 4321 904-648; tierseuchen@lsh.landsh.de

Denmark:

Contact Statens Serum Institut (e.g. Charlotte Kristiane Hjulsager at CKHJ@ssi.dk)