

Mass Mortality Caused by Highly Pathogenic Influenza A(H5N1) Virus in Sandwich Terns, the Netherlands, 2022

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We collected data on mass mortality in Sandwich terns (*Thalasseus sandvicensis*) during the 2022 breeding season in the Netherlands. Mortality was associated with at least 2 variants of highly pathogenic avian influenza A(H5N1) virus clade 2.3.4.4b. We report on carcass removal efforts relative to survival in colonies. Mitigation strategies urgently require structured research.

The 2021–2022 epidemic of highly pathogenic avian influenza (HPAI) A(H5N1) virus clade 2.3.4.4b has been unprecedented in terms of numbers of dead wild birds, species affected, spatial extent, and incidence in spring 2022 (1). Across Europe, multiple colony-breeding seabirds experienced HPAI

H5N1-associated mass mortalities during the breeding period, including the Sandwich tern (*Thalasseus sandvicensis*) (1,2). The Netherlands constitutes a major though vulnerable stronghold of the Sandwich tern in Europe; 15,000–20,000 breeding pairs have been documented across ≈10 colonies (<https://stats.sovon.nl/stats/soort/6110>). We sought to establish the scale of mortality occurring in Sandwich terns breeding in the Netherlands in 2022, characterize the associated HPAI H5N1 viruses and pathology, report on the carcass removal effort relative to survival, and investigate intracolony transmission dynamics.

The Study

We determined breeding colony locations and initial sizes in May 2022 through drone or ground counts (3). To establish breeding success and minimum estimates of mortality, we compiled data from late May through early July 2022 on numbers of live adults, chicks, fledglings, and late clutches in colonies, as well as on numbers of carcasses found in and around colonies, carcasses removed for destruction, and abandoned nests (Appendix 1 Table 1, <https://wwwnc.cdc.gov/EID/article/28/12/22-1292-App1.xlsx>). Sandwich terns lay 1–2 eggs and incubate for 21–29 days. Chicks fledge 25–30 days after hatching; annual fledging success is ≈0.5 per breeding pair (4–6). We used wild bird mortality databases to establish minimum estimates of adult mortality outside the colonies. Finally, we used data from the migration tracking website Trektellen (<https://www.trektellen.nl>) to compare the hourly averages of Sandwich tern passing rates at coastal observation points per week in 2022 to 2016–2021.

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We observed clinical signs and tested 44 carcasses for avian influenza virus by using a quantitative PCR to detect the influenza A virus matrix gene; we then followed up with subtype-specific PCRs on cloacal and tracheal swab specimens (7). We performed necropsy with histopathology and immunohistochemistry on 6 of the carcasses to establish cause of death. To study the relationship between viruses detected in Sandwich terns and other bird species, we determined full-genome sequences directly on swab RNA from 20 birds and submitted them to the GISAID database (<https://www.gisaid.org>), then compared

them to a sample of 57 other birds (Appendix 1 Table 2). We aligned sequences by using MAFFT version 7.475 (8), reconstructed phylogeny by using maximum-likelihood analysis with IQ-TREE software version 2.0.3 (9), and visualized the maximum-likelihood tree by using the R package ggtree (10).

To investigate HPAI H5N1 transmission dynamics within a breeding colony, we developed a susceptible-infectious-recovered model that included infection-fatality rate (IFR) and examined outcome as a function of IFR. IFR is the probability that a bird dies after infection, although in this study IFR also

Table. Mortality, fledgling success, carcass removal, and estimated fraction of Sandwich tern breeding population that died or disappeared in 10 breeding colonies, the Netherlands, 2022

Breeding colony no.	Date mortality first observed	Fledgling success (no. fledglings produced/no. initial breeding pairs)	Dead adults found	Dead chicks found	Abandoned eggs	Carcass removal	Estimated percentage of breeding population that died or disappeared from colony*
Colony 1	2022 May 30	1% (20/2,100)	342	425	Yes, all 700 late nests	Yes, with a 12-d delay, on 10 of next 18 d, adults and chicks	99%
Colony 2	2022 Jun 21	0% (0/150)	107	0	Yes, all 150 late nests	Once, with a 3-d delay, adults only	100%
Colony 3†	2022 Jun 4	0% (0/1,176)	170	Hundreds	Yes, many	Yes, with a 6-d delay, on 6 of next 18 d, adults only	100%
Colony 4†	2022 May 26	0.1% (5/3,374)	3,316	Thousands	Unknown	Twice, once 19 d after start, then 16 d later, adults only	99.8%
Colony 5	2022 May 29	0% (0/220)	400	0	Unknown	Once, 17 d after deaths started, adults only	100%
Colony 6	2022 May 31	Low (Few/3,016)‡	941	Thousands	Yes, all 245 late nests	Yes, without delay, on 15 of next 36 d, adults only	>92%
Colony 7	2022 May 31	11.1% (45/404)	115	Unknown	Yes, many of 40 late nests	Yes, without delay, on 14 of next 36 d, adults only	78%
Colony 8	2022 Jun 14	47.4% (65/137)	2	0	No (also no late nests)	No	5% or not applicable§
Colony 9	2022 Jun 6	9.5% (665/6,974)	2,368	3,122	No, there is activity above 400 late nests	Yes, with an 8-d delay, on 14 of next 27 d, adults and chicks	81%
Colony 10¶	2022 Jun 14	0% (0/600)	240	12	Unknown, 150 + 450 late nests	Yes, with a 7-d delay, on 3 of next 15 d, adults and chicks	100%
Overall		Low (Few/18,151 pairs)	8,001 (22% of breeding birds)	Thousands			

*Calculated by subtracting the ratio of fledgling success in 2022 over average fledgling success in previous years (0.5 fledgling per pair) from 100%.

†After colony 3 and colony 4 were decimated, a very late colony of 600 breeding pairs was established in July on Texel midway between these 2 colonies (53.022°N, 4.819°E), and this very late colony yielded 300 fledglings in September 2022.

‡Exact number not recorded.

§Because no carcass was examined, it is unclear if the 2 dead birds observed in this colony died from other causes or the cause of death was HPAI H5N1 but further infections in the colony were aborted.

¶In this colony, some very late birds did end up producing some fledglings (around 155) in August (Appendix 1 Table 1, <https://wwwnc.cdc.gov/EID/article/28/12/22-1292-App1.xlsx>).

includes birds leaving the colony. The model assumed a naive starting population, an infectious period of 5 days, and frequency-dependent transmission and considered only survivors of infection as recovered and total population size as not constant (Appendix 2, <https://wwwnc.cdc.gov/EID/article/28/12/22-1292-App2.pdf>).

Mass mortality was seen in 9 of the 10 Sandwich tern breeding colonies in 2022. In those colonies, out of a total of 18,151 breeding pairs, 8,001 adult Sandwich terns were found dead, and only a few chicks fledged (Video 1, <https://wwwnc.cdc.gov/EID/article/28/12/22-1292-V1.htm>; Video 2, <https://wwwnc.cdc.gov/EID/article/28/12/22-1292-V2.htm>). Only 1 small inland colony of 137 breeding pairs experienced no mass mortality and had a fledgling success rate (0.47 young/pair) consistent with previous years (0.50 young/pair) (Table; Figure 1, panel A). Outside of colonies, another 1,600 adult Sandwich

terns were reported dead between late May and end of June. The scale of mortality is reflected in the passage rate of Sandwich terns along the coast in May–June 2022 (Appendix 2 Figure 4).

Diseased birds were debilitated, unable to fly, and mostly lethargic, sometimes with wings spread out. At later stages, some displayed opisthotonos and occasionally flipped over backwards (Video 1, Video 3, <https://wwwnc.cdc.gov/EID/article/28/12/22-1292-V3.htm>; Appendix 2 Figures 5–10). We confirmed HPAI H5N1 virus infection in 23 of 24 dead Sandwich terns from colonies (the exception was a chick); infection was also confirmed in 20 of 20 birds outside of colonies (Appendix 2 Table 1). In the 4 necropsied PCR-confirmed adult birds, viral antigen expression was detected by immunohistochemistry in the pancreas ($n = 3$), duodenum ($n = 4$), or lung and nasal tissue ($n = 1$), colocalized with necrosis and inflammation (Appendix 2 Figure 18). Necropsy

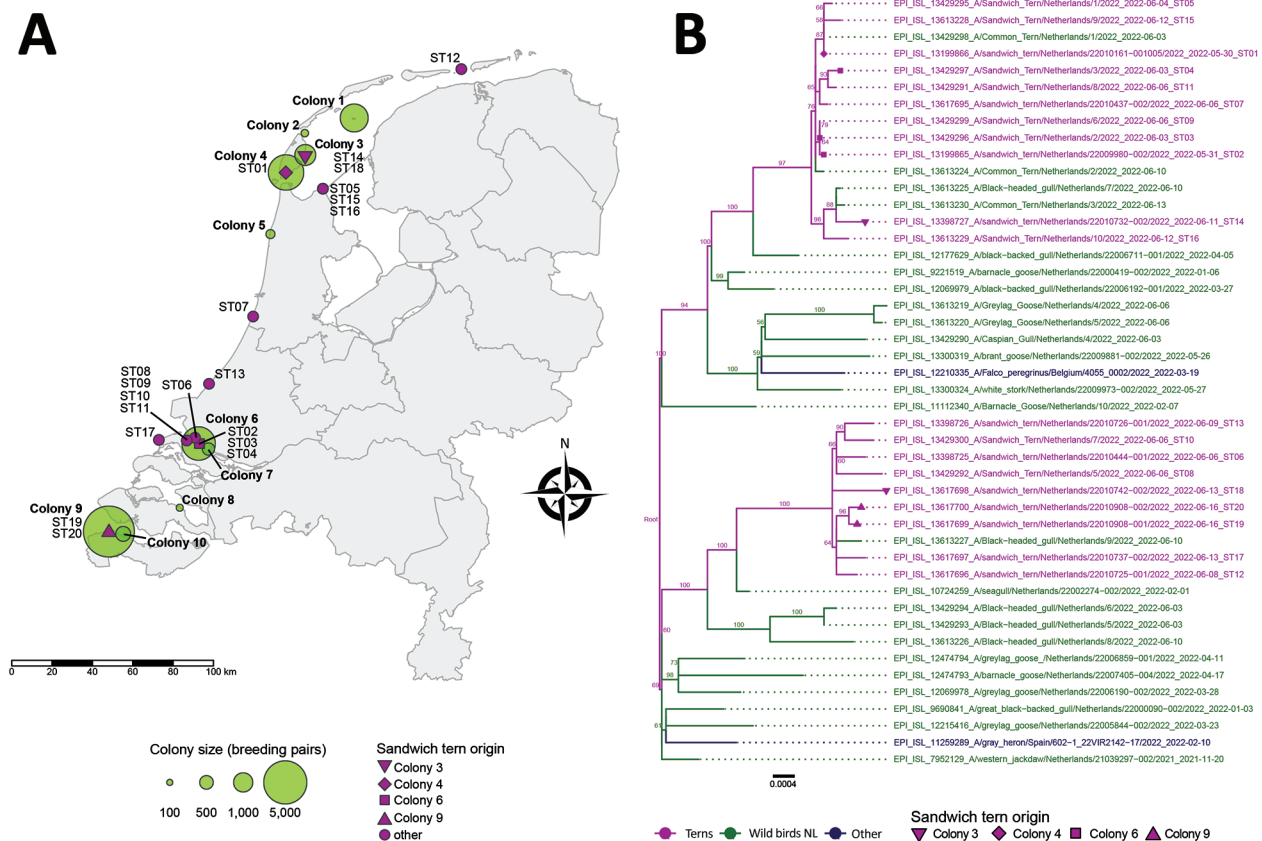


Figure 1. Location of Sandwich terns affected by locally acquired highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b viruses and phylogeny of viral segments, the Netherlands. A) Location and size (number of breeding pairs) of the Sandwich tern breeding colonies and the origin (finding location) of the Sandwich terns from which virus sequences ST01–ST20 shown in the phylogenetic tree in panel B were obtained. B) Maximum-likelihood tree (1,000 bootstraps) of the concatenated viral segments showing the H5N1 viruses detected in Sandwich terns together with viruses from other wild birds. Bootstrap values >50 are indicated at the branches. Identification numbers and symbols of the Sandwich terns correspond to those in the map, and the date that the bird was found dead is indicated. The GISAID sequences used in the phylogenetic analysis are listed in Appendix 1 Table 2 (<https://wwwnc.cdc.gov/EID/article/28/12/22-1292-App1.xlsx>).

findings and negative immunohistochemistry results in the 2 chicks we examined demonstrated that chick mortality was at least partly caused by starvation, likely after feeding was interrupted because of adult mortality (Appendix 2). This explanation was supported by field observations (Appendix 2 Figure 19).

Phylogenetic analysis demonstrated that the 20 fully sequenced viruses belonged to H5 clade 2.3.4.4b, and clustered with viruses detected in other wild bird species in the Netherlands, including in geese and gulls collected during January–April 2022 (Figure 1, panel B; Appendix 2). The Sandwich tern viruses clustered in 2 groups. Both variants were found in the northern and southern parts of the Netherlands and were even found within a single colony. These results suggest that at least 2 independent virus introductions into Sandwich terns occurred in the Netherlands, followed by transmission of both virus variants within and between breeding colonies.

Carcass removal effort was diverse (Table; Appendix 1, Table 1). In colonies with some survival, effort was overall more regular, frequent, and immediate or included chicks also, although survival might also have been affected by other undetermined factors.

In most colonies, a high proportion of the birds died or left (Table), indicating high IFR. The model shows that, even with a relatively low value of R_0 ($R_0 = 2$), at the higher end of the IFR range evidenced here, few of the birds remaining in the colony will have escaped infection at the end of the outbreak. Birds in the colony will have died or recovered and acquired immunity (Figure 2).

Conclusions

Our results substantiate that after Sandwich terns arrived in the Netherlands for breeding, HPAI H5N1 virus was introduced into their population at least twice. The virus then spread widely within and between breeding colonies, causing outbreaks that resulted in high adult and chick mortality in nearly all colonies. Infected birds probably died of systemic HPAI-associated disease, including acute pancreatic necrosis and duodenitis (11,12). Like other seabirds, Sandwich terns have low annual reproductive output but relatively long life-expectancy (2,4,6,13); therefore the effect of high adult mortality on population size could be seen for a long time. The Sandwich tern exemplifies how severely the continued circulation of HPAI H5N1 viruses in spring 2022 affected populations of colony-breeding birds without flock immunity in Europe (1).

Our study also demonstrates how outbreaks in breeding birds boosted virus propagation into the

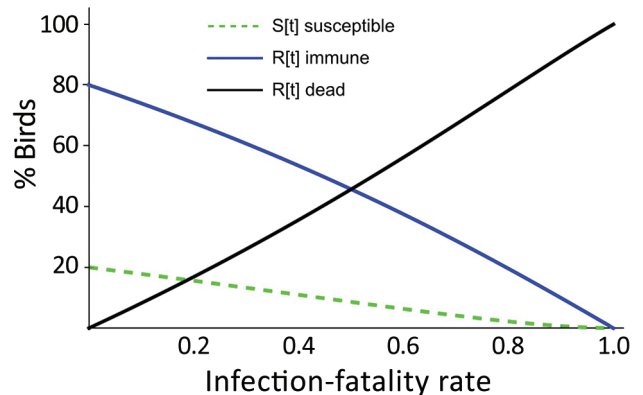


Figure 2. Model result for the introduction of highly pathogenic avian influenza (HPAI) A(H5N1) clade 2.3.4.4b viruses into a local Sandwich tern breeding colony population, the Netherlands. Graph demonstrates the distribution at the end of the local HPAI H5N1 outbreak, for dead (or departed) birds, escaping susceptible birds, and immune birds as a function of the infection-fatality rate (IFR), for a Sandwich tern population that was naive for HPAI H5N1 at the start of the local outbreak, for $R_0 = 2$. The model output indicates that the fraction of birds infected with HPAI H5N1, and hence also the fraction that dies, will increase with infection-fatality rate and that at a rate above 90%, virtually no more susceptible birds remain, only immune or dead (or departed) birds.

summer of 2022. The future involvement of Sandwich terns in HPAI endemicity can be evaluated once future population size and flock immunity have been analyzed from count data and serosurveillance. On the basis of our model, colony survivors would be mostly immune to HPAI.

Confirming HPAI as a major mortality factor in breeding colonies of Sandwich terns and other seabird species (2,14,15) underlines the paradigm shift to HPAI as a mortality factor of concern to wild species, in addition to poultry and humans. It stresses the importance of close international cooperation and data exchange to better understand and mitigate the global effect of HPAI on nature. More structured research on appropriate strategies to reduce massive propagation is urgently required. Carcass removal takes away a source of infection but might simultaneously enhance spread of infection and thus requires controlled study.

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Mass Mortality Caused by Highly Pathogenic Influenza A(H5N1) Virus in Sandwich Terns, the Netherlands, 2022

Appendix 2

Model

This section presents the model of the effect of infection-fatality rate (IFR) on HPAI H5N1 transmission dynamics in a breeding colony with an initially naive Sandwich tern (*Thalasseus sandvicensis*) population.

Specifics

This analysis focuses on the breeding colonies as clearly defined local populations. The birds in these breeding colonies are special in that they have a strong tendency to remain there to maximize their fitness. Breeding birds will disappear from a local population only because they die or because they leave after breeding failure.

As explained in the main article, the observed Sandwich tern mortality is considered to occur after introduction of the virus into a local breeding colony population consisting completely of naive individuals. The outbreaks are remarkable because a very high IFR appears to have occurred during these outbreaks, but the massive drops in numbers may partly be due to birds abandoning their nest after their partner and chicks died. The IFR is the probability that a bird dies when it has become infected but, in this model, it may also account for birds leaving the breeding colony population alive.

Model

The following Susceptible-Infectious-Recovered (SIR) model, which is a simplified but possibly relevant representation of the reality, looks at the outbreak in a local population (a breeding colony population of Sandwich terns) completely naive for the infecting H5N1 influenza strain, and considers the effect of IFR on the dynamics.

$$\frac{d S(t)}{dt} = -\beta \frac{S(t)I(t)}{S(t) + I(t) + R(t)}$$

$$\frac{d I(t)}{dt} = \beta \frac{S(t)I(t)}{S(t) + I(t) + R(t)} - \alpha I(t)$$

$$\frac{d R(t)}{dt} = (1 - IFR) \alpha I(t)$$

This formulation looks very much like the standard SIR model with the transmission term being divided by the population size assuming therefore frequency-dependent transmission (I). Two parts are special for this SIR model: 1) the total population size is not constant hence it is written as the sum of the numbers of susceptible birds, infectious birds and recovered birds, and 2) the recovered birds here are the only (surviving) birds that become immune as can be seen from the last equation.

Transmission (R_0) of this Highly Pathogenic Avian Influenza (HPAI) in Sandwich terns in a breeding colony does not need to be that high to obtain the observed pattern. We used a conservative value of $R_0 = 2.0$ here. Any other (higher) value would not have changed the result reported in the main text (see below). Observations on the final size as function of IFR would allow us to estimate the R_0 . The final size is the final outcome of the introduction of the virus to the local breeding colony population: i.e., the number of birds that died, that recovered and that remained susceptible (escaped infection). For example, in most of the 9 affected breeding colonies mortality (or in some cases departure) seemed to be near 100%, and in a few others around 80% (Table 1). The first value implies $IFR = 1.0$ (100%) and could be any $R_0 > 1$. The second value implies for $R_0 = 2.0$ that $IFR = 0.8156$ (82%) and apart from the 80% dead or otherwise disappeared birds, most of the surviving birds will be immune (19%) and the remaining 1% of birds will have escaped infection. For higher values of R_0 all surviving birds will also be infected and thus will be recovered and immune.

If $IFR = 0$ than for the parameters chosen here, i.e., $R_0 = 2.0$ and the duration of the infectious period is 5 days (thus, $\beta = 0.4 \text{ day}^{-1}$ and $\alpha = 0.2 \text{ day}^{-1}$) on average 20% will escape infection in a major outbreak (Appendix 2 Figure 1) and the remaining 80% will become immune.

If $IFR = 1$, i.e., 100% mortality if infected, then with the same parameters more individuals will become infected (here all individuals as $IFR = 1$) and hence also more individuals will die. Given $IFR = 1$ all individuals will die (Appendix 2 Figure 2).

In the main article the Final Size for the different type of birds as a function of IFR is given for $R_0 = 2.0$. Choice of a higher value for R_0 will only change the fraction of the surviving birds that is immune rather than escaped infection. For example, for $R_0 = 6.0$ the figure in the main text would look like Appendix 2 Figure 3.

Discussion of Assumptions

The model used has the following assumptions, each followed by the argumentation why this assumption was used:

1. Not stochastic: the calculation just shows the average. In the situation considered here IFR is almost 1.0, the average of close to 100% infected and also close to 100% dying there is little variation even in the stochastic model. As in 9 of 10 breeding colonies that seems what has happened the calculation seems in agreement with the data. Because after dying of the original breeding birds, other conspecific birds will attempt to establish themselves and thus one cannot distinguish whether mortality is 100% or close to 100%.

2. Survival of the virus in the environment is not taken into account: in the model the recovery rate parameter $\alpha = 0.2 \text{ day}^{-1}$: individuals are expected to be infectious for on average 5 days ($1/0.2$) with an exponential distribution. In reality birds may die earlier but infectious material, i.e., virus, will be present in the environment. In a similar model for breeding black-headed-gulls, a similar value, i.e., 0.26 day^{-1} , was used for the exponential decay rate parameter (Verhagen, Fouchier, and de Jong in prep.).

3. Crucial assumption is that the transmission rate is frequency dependent which means that the contact rate is constant for different population sizes. Here the population size decreases and thus for the remaining birds the chances of encountering the infectious birds and infectious material (shed before death or dead birds) increase. This is the case because with high IFR all birds in a population are either susceptible or infectious and not immune individuals. This seems a reasonable assumption, as all the area where the breeding birds have died will have a lot of infectious material and breeding birds will continue taking care of the nest and the chicks in this contaminated environment.

4. The population was initially immunologically naïve for HPAI H5N1. This assumption is based on the lack of reports on HPAI die-offs in Sandwich terns in previous years (2).

Passing Rate

The scale of Sandwich tern mortality is reflected in the decreased hourly average passing rate at coastal observation points in the Netherlands, June through August 2022, as compared to 2016–2021 (Appendix 2 Figure 4). It should be noted that observation effort (hours) is not equally distributed along the coast and that locations near hard-hit colonies may be overrepresented in this graph. Source data: www.trektellen.nl (3).

Clinical Signs, Postmortem Postures, and Outbreak Features

Diseased birds were debilitated, unable to fly, mostly lethargic, sometimes with wings spread out. At later stages some displayed opisthotonos, while occasionally flipping over backward, sometimes dying in that position (Appendix 2 Figures 4-14). Necrophilia may have enhanced transmission among adults (Appendix 2 Figure 15). Carcass removal around and away from colonies required organization and came with logistical challenges (Appendix 2 Figures 16, 17).

PCR Test Results

Overview of the dead Sandwich terns tested for avian influenza by PCR, with outcome and virus sequence number if sequencing performed (Appendix 2 Table 1). Those also submitted for necropsy with histopathology and immunohistochemistry are indicated.

Pathology and Immunohistochemistry

Here we present the results of the necropsies with histopathology and immunohistochemistry on 4 adult and 2 chick Sandwich terns.

Adults

Four dead adult Sandwich terns, 1 from Engelsmanplaat and 3 from Breeding Colony 3 (Wagejot), were evaluated for pathologic changes and tissue related virus protein expression. All

four birds had tested positive for HPAI H5N1 by PCR-tests, and 2 birds were also sequenced (Appendix 2 Table 2). Gross pathology showed the carcasses were moderately autolytic. All birds were in a good body condition; however, the stomach was empty, and the intestinal tract was poorly filled. The livers were enlarged, extending 2–3 cm below the carina. Spleens were not enlarged (0.5cm x 0.5cm). (Appendix 2 Table 3). Histopathology and immunohistochemistry showed the most prominent finding in all birds was the severe acute pancreas necrosis (Appendix 2 Figure 18, panel A) with associated viral antigen expression in cells (Appendix 2 Figure 18, panel B), which was present in 3 of the 4 birds. All birds showed a mild to moderate non-suppurative duodenitis (Appendix 2 Figure 18, panel C), which was also associated with viral antigen expression (Appendix 2 Figure 18, panel D). The lungs showed mainly a-specific changes, such as edema and congestion; only in one bird was there mild virus expression in the lung and nasal/sinus tissue. There was no evidence for infiltrates of inflammatory cells or virus expression in the cerebrum and cerebellum, and also the other organs showed no significant histopathologic findings (nor virus expression), which could be associated with another (infectious) cause of death (Appendix 2 Tables 3, 4).

By this we conclude that the HPAI infection in the examined adult Sandwich terns was associated with severe pancreas necrosis and duodenitis and less prominent changes in the respiratory tract and other investigated organs. Acute pancreas necrosis has also been reported in other bird species infected with this virus clade (4).

Chicks

Two dead Sandwich tern chicks from Breeding Colony 6 (Slijkplaat) were evaluated for pathologic changes and tissue related viral antigen expression. The larger chick had tested positive for HPAI H5N1 by PCR-tests, while the smaller had tested negative (Appendix 2 Table 2). Gross pathology showed the stomachs were empty (Appendix 2 Table 3). Histopathology and immunohistochemistry showed the lesions in the chicks were not associated with viral antigen expression. At least in the smaller chick, death by dehydration/starvation could be probable (Appendix 2 Tables 3, 4).

Field Observations on Timing of Adult Mortality Versus Chick Mortality

Field observations showed live chicks among dead adults (Appendix 2 Figure 12). Counts of dead adults and dead chicks over time support pathology that indicates that chick mortality was at least partly due to starvation following interrupted feeding because of adult HPAI-H5N1-associated mortality (Appendix 2 Figure 19).

Phylogentic Trees

The maximum-likelihood (ML) tree of the 8 virus segments PB2, PB1, PA, HA, NP, NA, MP and NS are represented in Appendix 2 Figures 20–27.

References

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4. Lean FZX, Vitores AG, Reid SM, Banyard AC, Brown IH, Núñez A, et al. Gross pathology of high pathogenicity avian influenza virus H5N1 2021-2022 epizootic in naturally infected birds in the United Kingdom. One Health. 2022;14:100392. [PubMed](https://doi.org/10.1016/j.onehlt.2022.100392) <https://doi.org/10.1016/j.onehlt.2022.100392>

Appendix 2 Table 1. Overview of dead Sandwich terns tested for avian influenza by PCR with outcome and virus sequence number if sequencing was performed

Date found	Location found	Type	X	Y	Number tested for AI by PCR	Number H5N1 positive in PCR	Virus sequence number
30 May '22	Colony 4	Colony outskirts bird	4.763	53.996	5	5	ST01
31 May '22	Colony 6*	Colony bird	4.154	51.799	7	6†	ST02
3 June '22	Colony 6	Colony bird	4.154	51.800	3	3	ST03-ST04
4 June '22	Den Oever	Away from colony	5.030	52.941	1	1	ST05
5 June '22	Maasvlakte	Away from colony	3.981	51.927	1	1	
5 June '22	Strand Ijmuiden	Away from colony	4.556	52.452	1	1	
6 June '22	Hellevoetsluis	Away from colony	4.119	51.826	1	1	ST06
6 June '22	Zandvoort	Away from colony	4.527	52.369	1	1	ST07
6 June '22	Stellendam	Away from colony	4.060	51.813	4	4	ST08-ST11
6 June '22	Colony 4	Colony outskirts bird	4.786	53.004	1	1	
7 June '22	Colony 4	Colony outskirts bird	4.755	53.011	1	1	
8 June '22	Egelsmanplaatt‡	Away from colony	6.059	53.472	1	1	ST12
8 June '22	Renesse	Away from colony	3.722	51.739	3	3	
9 June '22	Den Haag	Away from colony	4.214	52.067	1	1	ST13
11 June '22	Colony 3§	Colony bird	4.898	53.086	3	3	ST14
12 June '22	Den Oever	Away from colony	5.030	52.941	2	2	ST15-ST16
13 June '22	Ouddorp	Away from colony	3.860	51.813	3	3	ST17
13 June '22	Colony 3	Colony bird	4.897	53.087	2	2	ST18
15 June '22	Den Andel	Away from colony	6.525	53.424	1	1	
16 June '22	Colony 9	Colony bird	3.518	51.400	2	2	ST19-ST20
Totals					44	43	

*Necropsy with histopathology and immunohistochemistry performed on 2 chicks, 1 PCR-positive and another PCR-negative. AI, avian influenza.

†The negative case was one of the necropsied chicks.

‡Necropsy with histopathology and immunohistochemistry on 1 adult bird.

§Necropsy with histopathology and immunohistochemistry performed on 3 adult birds.

Appendix 2 Table 2. Identification information and the results of the virological analysis for the 4 adult and 2 chick Sandwich terns submitted for necropsy

Id	Age group	Location found	Date found dead	Date necropsied	M-PCR trachea (Ct*)	M-PCR cloaca (Ct)	H5N1 subtype	Sequence number
A1	Adult	Engelsmanplaat	8 June '22	14 June '22	30.27	23.15	H5N1	ST12
A2	Adult	Colony 3 (Wagejot)	11 June '22	14 June '22	20.99	18.72	H5N1	ST14
A3	Adult	Colony 3 (Wagejot)	11 June '22	14 June '22	23.66	22.99	H5N1	
A4	Adult	Colony 3 (Wagejot)	11 June '22	14 June '22	23.05	20.52	H5N1	
C1	Chick (large)	Colony 6 (Slijkplaat)	31 May '22	1 June '22	35.75	25.75	H5N1	
C2	Chick (small)	Colony 6 (Slijkplaat)	31 May '22	1 June '22	40.20	Nd†	Nd	

*Ct, cycle threshold.

†Nd, not detectable.

Appendix 2 Table 3. Gross pathology and histopathology results for the 4 adult and 2 chick Sandwich terns submitted for necropsy*

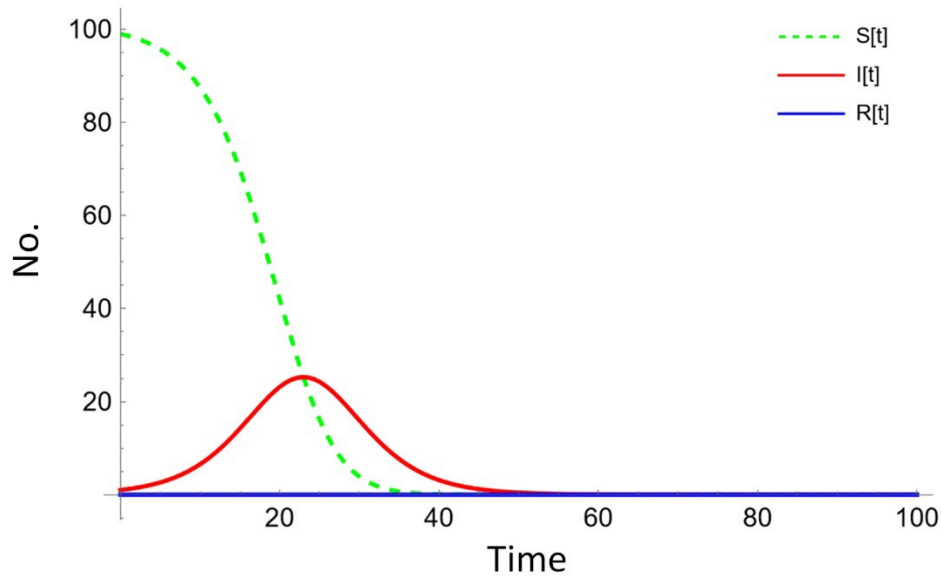
Id	Sex	Condition	Stomach contents	Other	Histopathologic summary
A1	M	Normal	Empty	No changes in the pancreas or the brain. Spleen not enlarged.	Mild hyperemia in cerebrum and cerebellum, no infiltrates. Lung edema, pneumoconiosis, possibly edema in the parabronchial lumen and air trapped in capillary lumen. Spleen np†. Other organs severe autolysis.
A2	F	Normal	Empty	No changes in the pancreas or the brain. Spleen not enlarged.	Mild hyperemia in cerebrum and cerebellum, no infiltrates. Hyperemic lungs, edema in the parabronchial lumen, pneumoconiosis. Focal extensive acute necrosis in the pancreas. Spleen np. Uric acid congestion in tubular lumina of kidney with minimal mineralization. Ovarium no lesions.
A3	F	Normal	Empty	No changes in the pancreas or the brain. Spleen not enlarged.	Possibly infiltrates in glandular tissue next to eye. Mild hyperemia in cerebrum and cerebellum, no infiltrates. Hyperemic lungs, edema in the parabronchial lumen, pneumoconiosis. Non suppurative enteritis duodenum. Extensive acute necrosis of the exocrine pancreas. Liver multifocal necrosis. Spleen np. Ovarium no lesions.
A4	M	Normal	Empty	No changes in the pancreas or the brain. Spleen not enlarged.	Necrosis, hemorrhage, and round nucleated cells with fibrin in glandular nasal/sinus tissue. Mild hyperemia in cerebrum and cerebellum, no infiltrates. Lung edema in the parabronchial lumen, pneumoconiosis, non-suppurative infiltrates interstitially. Extensive acute necrosis of the exocrine pancreas. Few lymphocytes in the lamina propria in the duodenum. Spleen autolysis with mixed population of lymphocytic and heterophilic granulocytes, few macrophages with hemosiderin. Adult trematodes in the collecting tubes of the kidney.
C1	Nd	Nd	Empty	Juvenile bird	Subepithelial lymphoid infiltrates in sinus, acute fibrinous pneumonia, non-suppurative gastritis, other organs severe autolysis.
C2	Nd	Nd	Empty	Juvenile bird	Necrotic heterophilic epidermitis next to beak, subepithelial lymphoid and heterophilic infiltrates in sinus, hepatic vacuolization, spleen np, other organs no significant changes.

*Nd, not detectable; Np, not present.

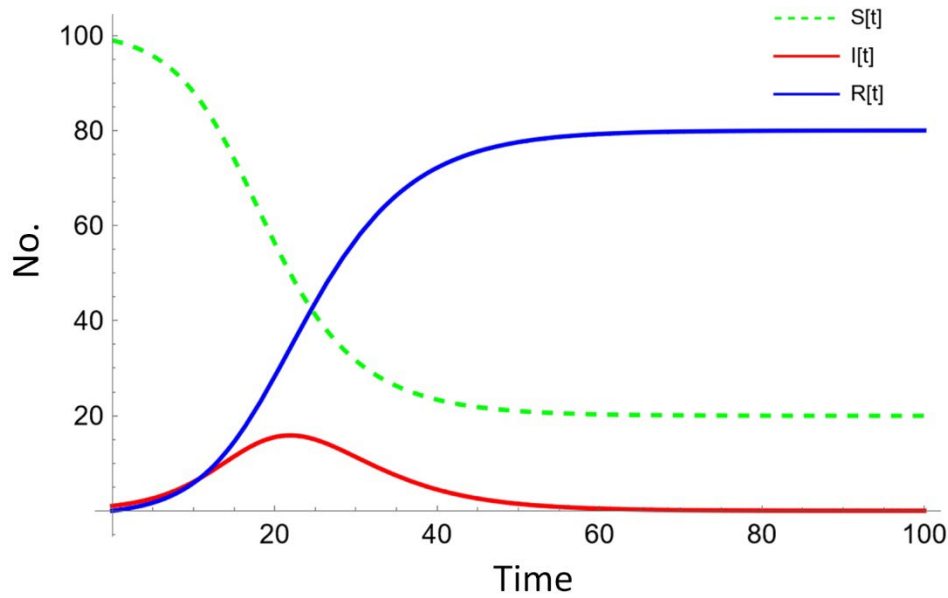
Appendix 2 Table 4. Immunohistochemistry results for the 4 adult and 2 chick Sandwich terns submitted for necropsy*

Id	Duodenum	Pancreas	Nasal/Sinus tissue	Lung	Brain	Other
A1	1	1	Np	0	0	0
A2	1	0	0	0	0	0
A3	1*	1*	0	0	0	0
A4	1	1	1	1	0	0
C1	0	0	0	0	0	0
C2	0	0	0	0	0	0

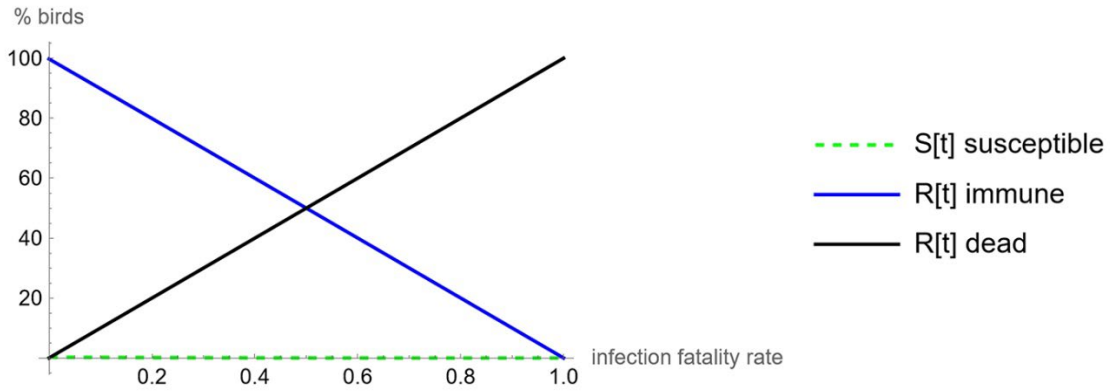
*Included in Appendix 2 Figure 18. Np, not present.



Appendix 2 Figure 1. The dynamics of the infection in a local population with infection-fatality rate = 0.0. S are susceptible (naïve) birds, I are infected birds, and R are recovered (immune) birds.



Appendix 2 Figure 2. The dynamics of the infection in a local population with infection-fatality rate = 1.0. S are susceptible (naïve) birds, I are infected birds, and R are recovered (immune) birds.



Appendix 2 Figure 3. The final size for $R_0 = 6.0$



Appendix 2 Figure 4. Alongshore movements of Sandwich terns at coastal observation points in the Netherlands in 2022 (until August 10, 2022) compared to 2019–2021 and 2016–2018.



Appendix 2 Figure 5. Diseased Sandwich terns unable to fly away. HPAI H5N1 outbreak, Breeding Colony 4 (Petten) outskirts, June 12, 2022. Photo by Susanne Kühn.



Appendix 2 Figure 6. Diseased lethargic and dead Sandwich terns. HPAI H5N1 outbreak, Breeding Colony 4 (Petten) outskirts, June 12, 2022. Photo by Susanne Kühn.



Appendix 2 Figure 7. Diseased lethargic Sandwich tern. HPAI H5N1 outbreak, Breeding Colony 4 (Petten) outskirts, June 12, 2022. Photo by Susanne Kühn.



Appendix 2 Figure 8. Diseased lethargic Sandwich tern, wings hanging, respiratory distress. HPAI H5N1 outbreak, away from colonies, June 14, 2022. Photo by Sander J. Lilipaly.



Appendix 2 Figure 9. Diseased lethargic Sandwich tern, unable to flee the cat. HPAI H5N1 outbreak, vicinity of Breeding Colony 2 (Steenplaat), June 18, 2022. Photos by Wilma Booij.



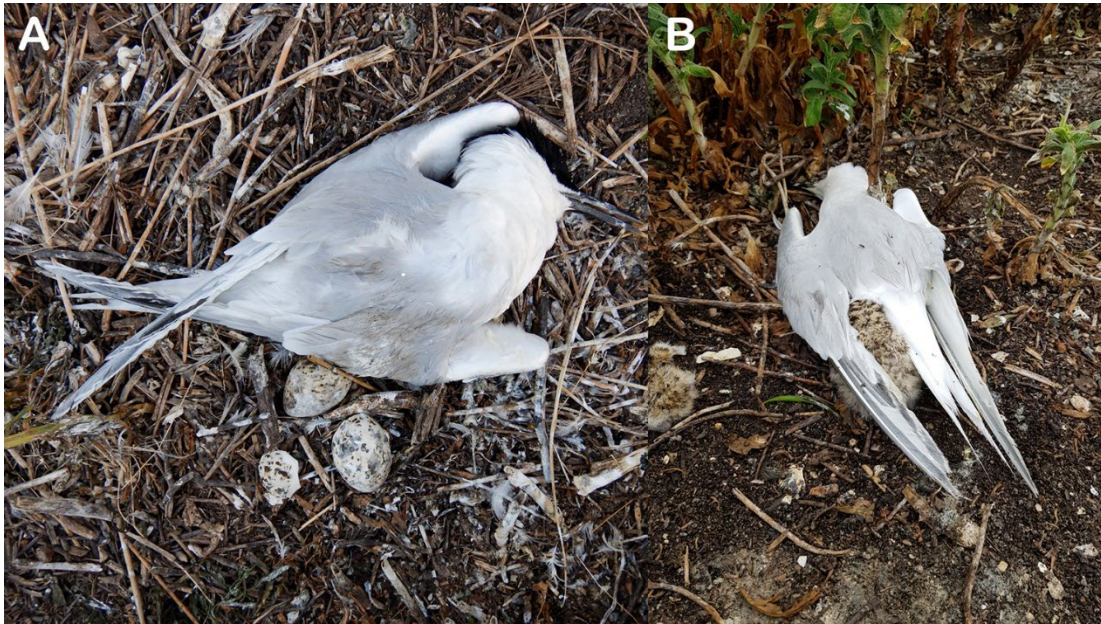
Appendix 2 Figure 10. Diseased Sandwich tern fledgling, unable to flee, wings hanging wide aside. HPAI H5N1 outbreak, Breeding Colony 6 (Slijkplaat), June 6, 2022. Photo by Mónica Z. Ballmann.



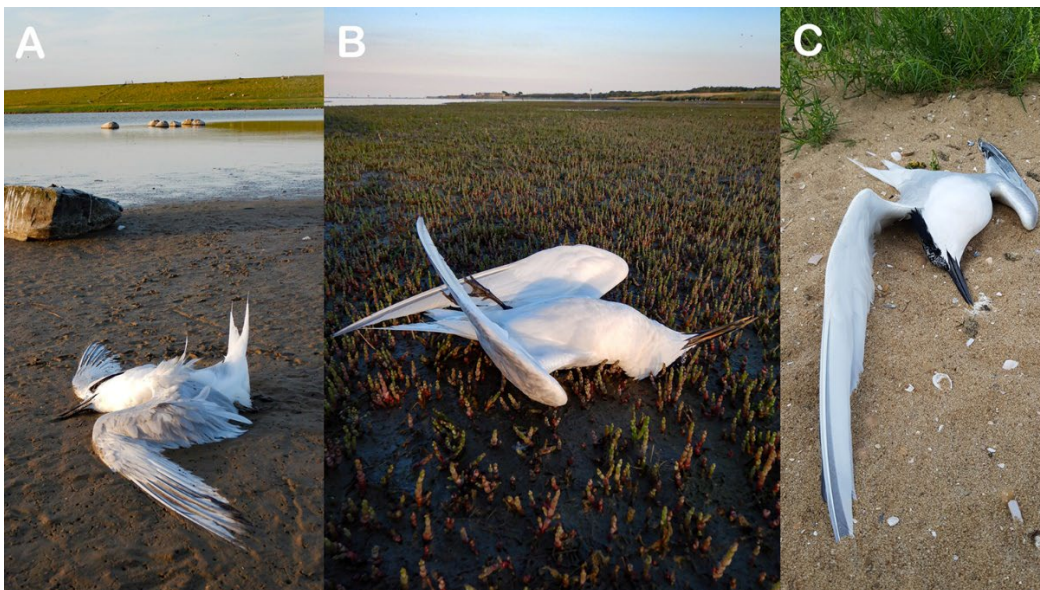
Appendix 2 Figure 11. Sandwich terns, breeding among the dead. HPAI H5N1 outbreak, Breeding colony 9 (Waterdunen), June 2022. Webcam image Bureau Waardenburg/Het Zeeuws Landschap.



Appendix 2 Figure 12. Sandwich terns dead on nests, some live chicks (these sometimes then starved to death – see pathology). HPAI H5N1 outbreak, Breeding Colony 6 (Slijkplaat), June 4, 2022. Photo by Ronald in 't Veld.



Appendix 2 Figure 13. Sandwich terns, dead on nest. HPAI H5N1 outbreak. A) Breeding Colony 3 (Wagejot), June 15, 2022. B) Breeding Colony 6 (Slijkplaat), June 6, 2022. Photo A by Susanne Kühn, photo B by Mónica Z. Ballmann.



Appendix 2 Figure 14. Dead Sandwich terns with tail still in opisthotonos position (A), birds in flipped over distorted position (B, C). HPAI H5N1 outbreak, Breeding colony 4 (Petten) outskirts, June 14, 2022 (A) and June 17, 2022 (B) and Breeding Colony 6 (Slijkplaat), June 10, 2022 (C). Photos A and B by Susanne Kühn, photo C by Mónica Z. Ballmann.



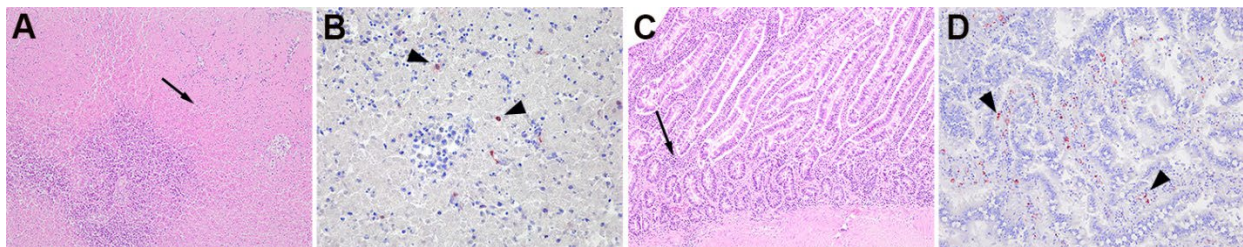
Appendix 2 Figure 15. Necrophilia may have enhanced transmission. HPAI H5N1 outbreak, Breeding Colony 3 (Wagejot), June 5, 2022. Photo by Mardik Leopold.



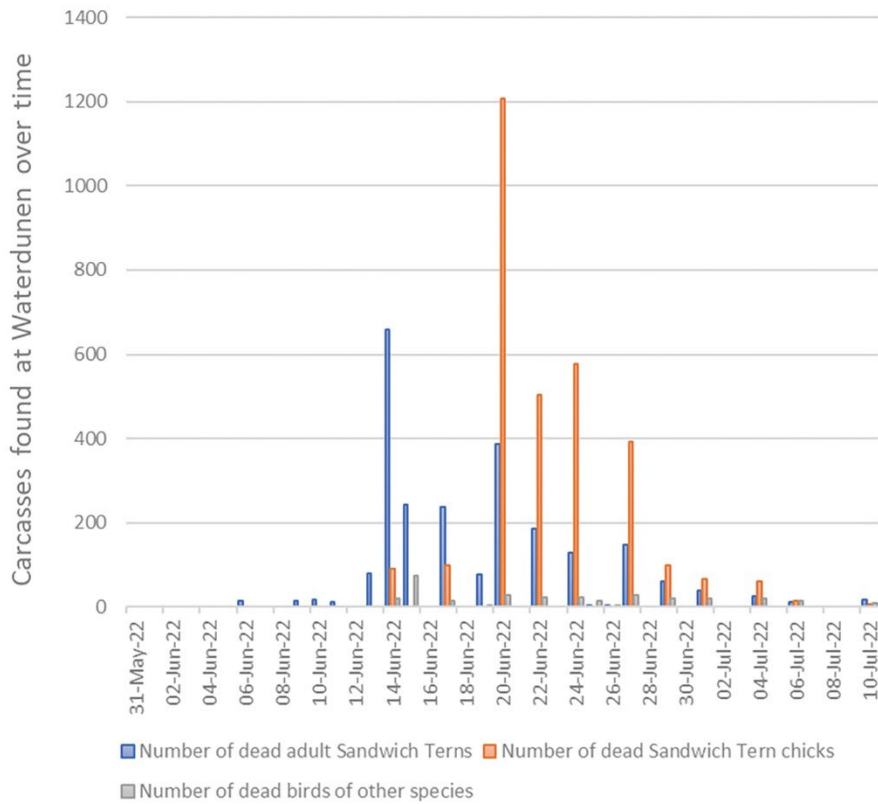
Appendix 2 Figure 16. Sandwich tern carcass removal. HPAI H5N1 outbreak, outskirts of Breeding Colony 4 (Petten), 4 June 4, 2022. Photo by Marc Plomp.



Appendix 2 Figure 17. Sandwich tern carcass removal. HPAI H5N1 outbreak, Breeding Colony 10 (Hooge Platen), June 21, 2022 (A) and away from colonies (Scheelhoek), June 15, 2022. Photo A by Fred Schenk, photo B by Mónica Z. Ballmann.



Appendix 2 Figure 18. HPAI related histopathology and viral protein expression: A) Extensive severe acute pancreas necrosis with loss of cellular detail (arrow) evaluated with hematoxylin and eosin (HE) stain, objective 20x; B) multifocal positive staining of individual epithelial cells in pancreas (arrowhead), immunohistochemistry (IHC), objective 40x; C) mild to moderate lymphoplasmacytic duodenitis (arrow) with viral antigen expression in epithelial cells (arrowhead); D), objective 20x.

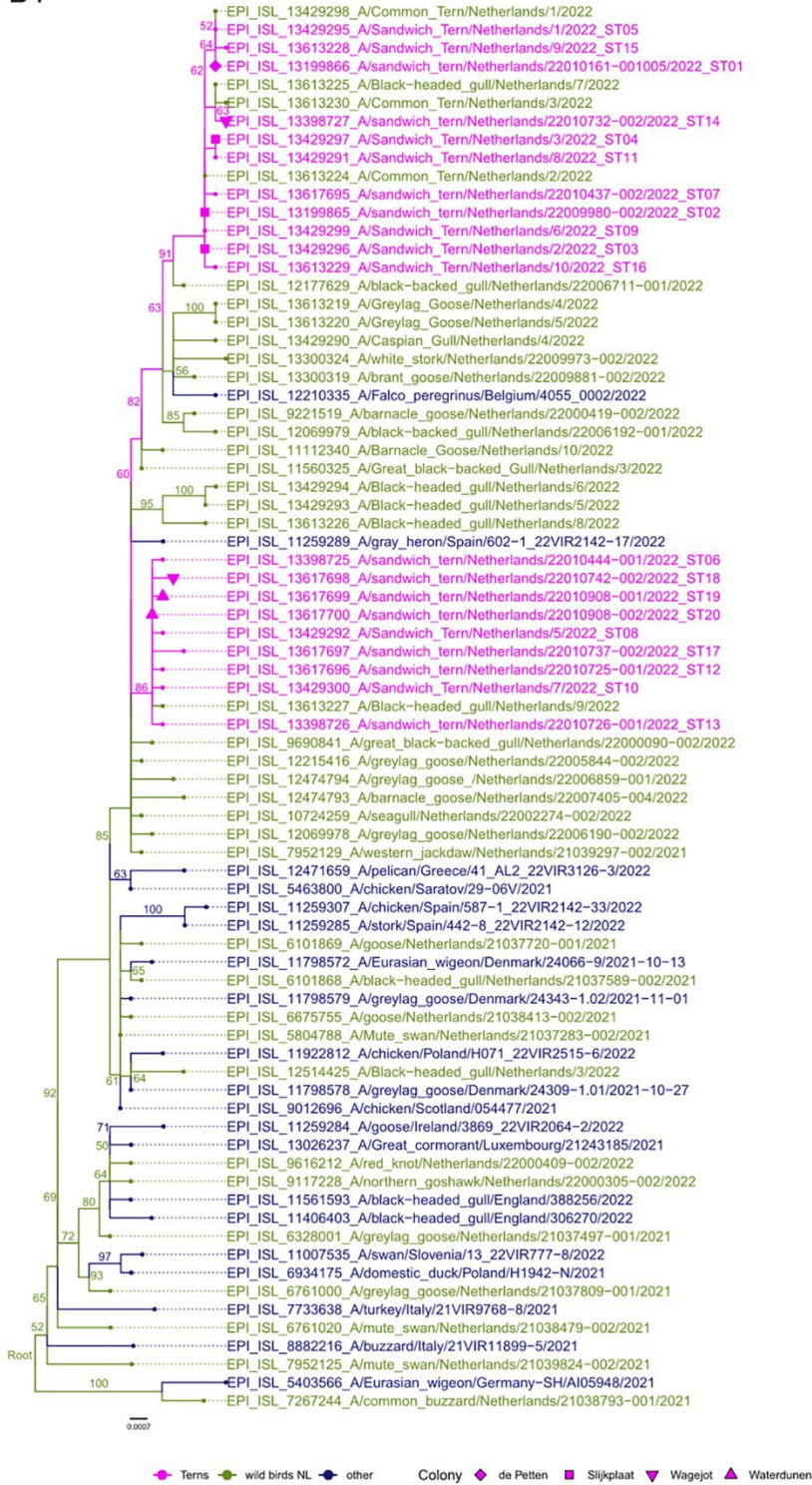


Appendix 2 Figure 19. Adult Sandwich tern mortality peaked before chick mortality in Breeding Colony 9 (Waterdunen).



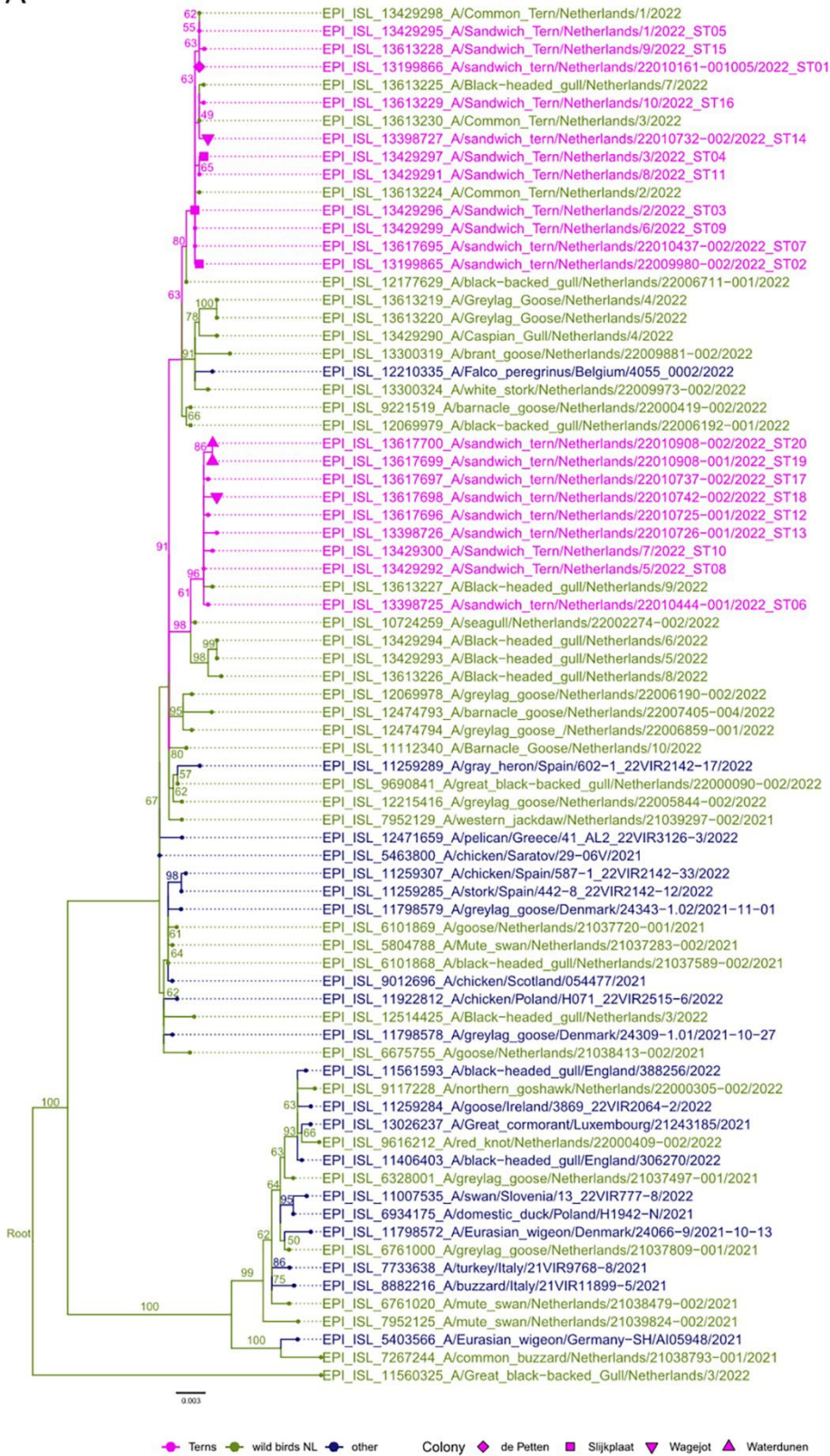
Appendix 2 Figure 20. The maximum-likelihood (ML) tree for PB2

PB1



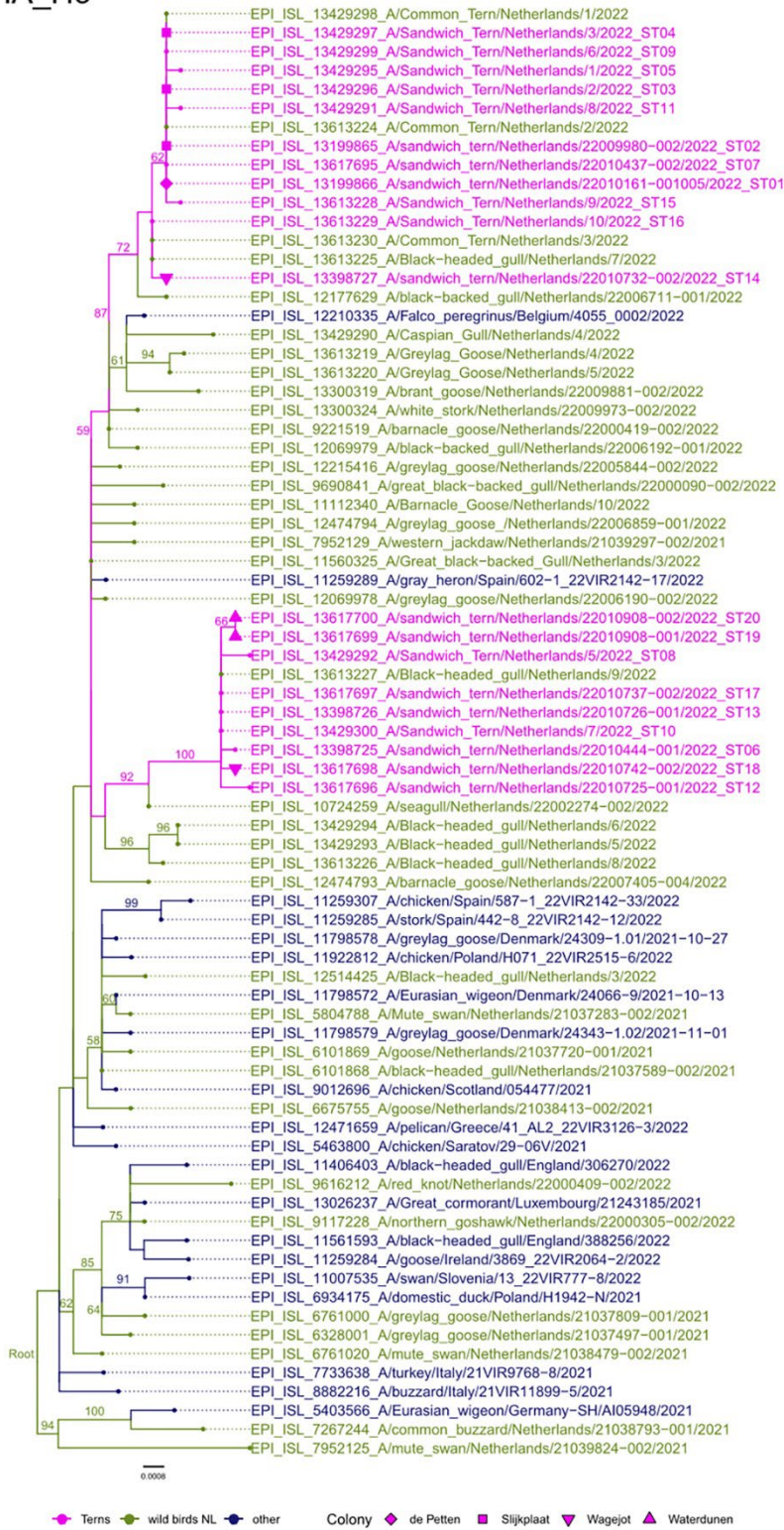
Appendix 2 Figure 21. The maximum-likelihood (ML) tree for PB1

PA



Appendix 2 Figure 22. The maximum-likelihood (ML) tree for PA

HA_H5



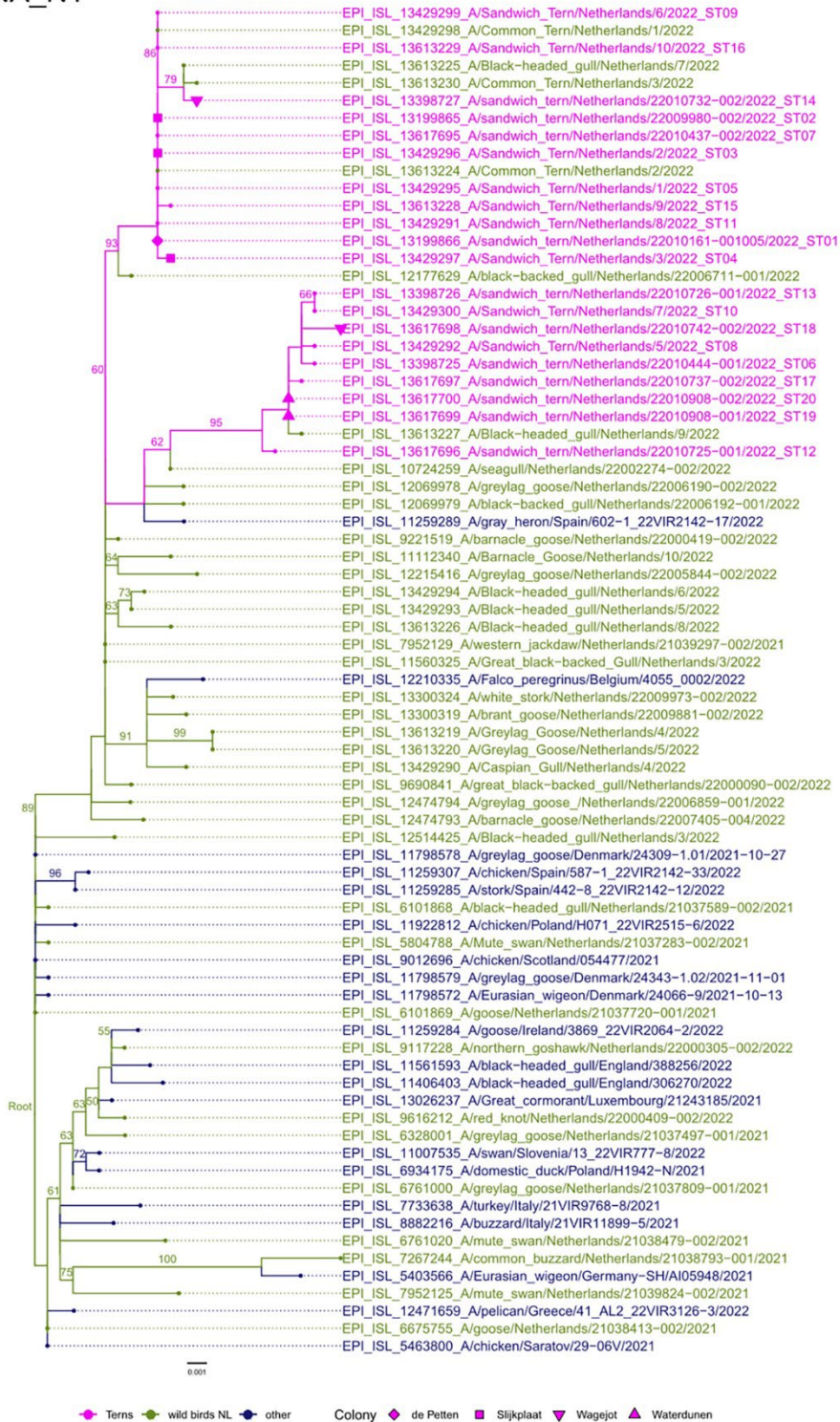
Appendix 2 Figure 23. The maximum-likelihood (ML) tree for HA

NP



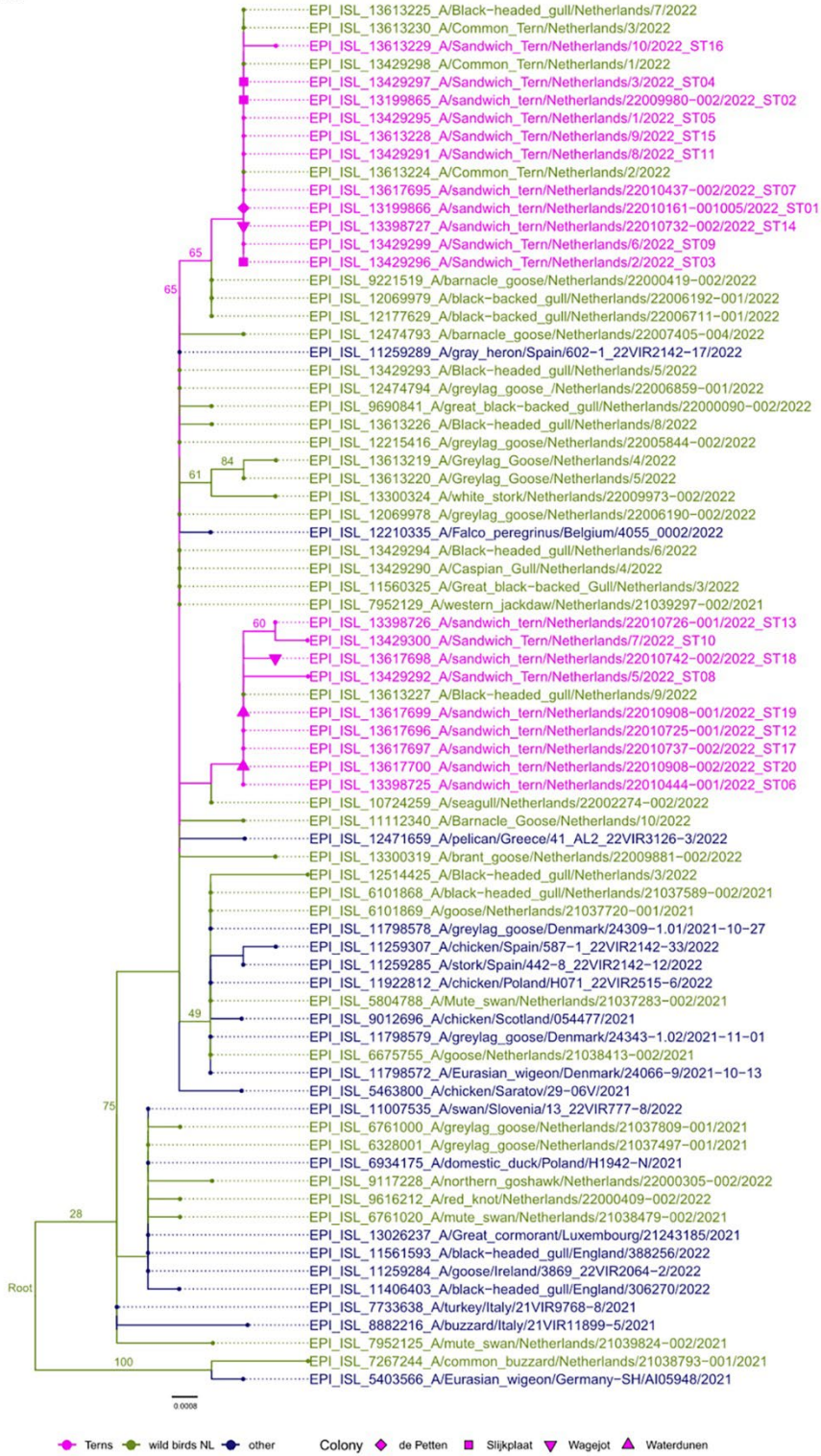
Appendix 2 Figure 24. The maximum-likelihood (ML) tree for NP

NA_N1



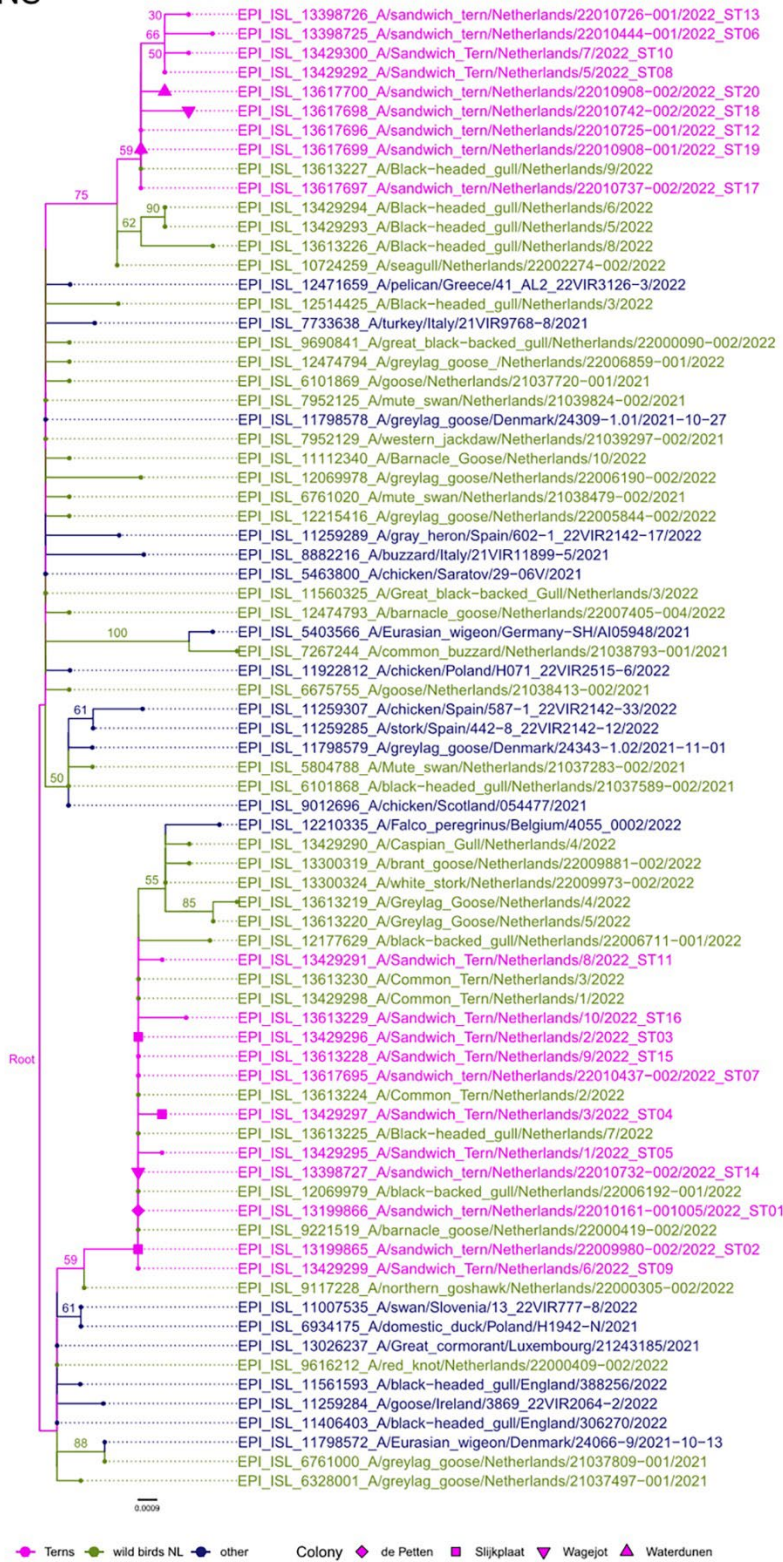
Appendix 2 Figure 25. The maximum-likelihood (ML) tree for NA

MP



Appendix 2 Figure 26. The maximum-likelihood (ML) tree for MP

NS



Appendix 2 Figure 27. The maximum-likelihood (ML) tree for NS