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High pathogenicity avian influenza virus H5N1 infection in skua and gulls in the United Kingdom, 2022

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	detected subsequently. Microscopical analysis revealed neuro-, pneumo-, lymphoid- and cardiotropism of HPAIV H5N1, with the most common virus-associated pathological changes being pancreatic and splenic necrosis. Examination of the reproductive tract of the great skua revealed HPAIV-associated oophoritis and salpingitis, and virus replication within the oviductal epithelium. The emergence of HPAIV in seabirds Stercorariidae and Laridae, particularly during summer 2022, has challenged the dogma of HPAIV dynamics, posing a significant threat to wild bird life with potential implications for the reproductive performance of seabirds of conservation importance.

Highly pathogenic avian influenza virus H5N1 infection in skua and gulls in the United Kingdom, 2022

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Abstract

The re-emergence of the highly pathogenic avian influenza virus (HPAIV) subtype H5N1 in the United Kingdom in 2021-2022 has caused unprecedented epizootic events in wild birds and poultry. During the summer of 2022, there was a shift in virus transmission dynamics resulting in increased HPAIV infection in seabirds and

consequently a profound impact on seabird populations. To understand the pathological impact of HPAIV in seabirds, we evaluated the virus antigen distribution and associated pathological changes in the tissues of great skua (*Stercorarius skua*, n=8), long tailed skua (*Stercorarius longicaudus*, n=1), European herring gull (*Larus argentatus*, n=5), and black-headed gull (*Chroicocephalus ridibundus*, n=4), which succumbed to natural infection of HPAIV during the summer of 2022. Cases were collected from Shetland, including Scatness (mainland), No Ness (mainland), Clumlie (mainland), Hermaness (island), Fair Isle (island), Noss (island), and the West Midlands, South East, and South West of England. Grossly, gizzard ulceration was observed in one great skua and pancreatic necrosis was observed in four herring gulls, with intra-lesional virus antigen detected subsequently. Microscopical analysis revealed neuro-, pneumo-, lymphoid-, and cardiomyotropism of HPAIV H5N1, with the most common virus-associated pathological changes being pancreatic and splenic necrosis. Examination of the reproductive tract of the great skua revealed HPAIV-associated oophoritis and salpingitis, and virus replication within the oviductal epithelium. The emergence of HPAIV in seabirds Stercorariidae and Laridae, particularly during summer 2022, has challenged the dogma of HPAIV dynamics, posing a significant threat to wild bird life with potential implications for the reproductive performance of seabirds of conservation importance.

Keywords

Highly pathogenic avian influenza virus, H5N1, multisystemic tropism, pancreatic necrosis, splenic necrosis, reproductive pathology

Highly pathogenic avian influenza virus (HPAIV) H5N1 clade 2.3.4.4b Goose/Guangdong (Gs/Gd) lineage has re-emerged in Europe during the 2020-2021, 2021-2022, and 2022-2023 seasons (defined as start of each October) and has brought about a series of epizootic events in poultry and wild birds. The re-emergence of HPAIV H5N1 clade 2.3.4.4.b in Europe and the United Kingdom (UK) during 2021-2022 has also contributed to the trans-Atlantic dissemination of virus into North America likely mediated though migratory wild birds.[13]

Conventionally, it is understood that Anseriformes are the carrier for HPAIV during the winter period in Western Europe. However during the 2021-2022 HPAIV season in the UK, there was a shift in infection from Anseriformes predominating in the colder months to a series of explosive outbreaks in seabird species across the northern coast of Scotland during the summer.[5] During the summer of 2021, infection with H5N1 was detected in great skuas (*Stercorarius skua*)[7] but those events, alongside sporadic small-scale outbreaks across northern Europe were the only cases of H5N1 reported during the summer months. In contrast, during the summer of 2022, infection in great skuas was detected several months earlier than during 2021 and was followed by extensive outbreaks in a number of shorebird species (Order Charadriiformes).[16] High mortality events in seabirds, including northern gannet, great skua, and several species of gulls, were observed.[5,16] Seabirds from the Laridae family have been previously associated with infection with low pathogenic avian influenza virus (LPAIV).[18,21,25,35,57] However, a recent experimental model demonstrated that previous exposure of the European herring gulls (*Larus argentatus*) with LPAIV H5N1 (non-Gs/Gd) or H13N6 only confers partial protection to a subsequent HPAIV H5N8 clade 2.3.4.4b challenge.[53]

Prior to the unusual increase in cases during the summer of 2022, HPAIV-associated disease in the Laridae has been sporadically reported in East Asia and Europe, often in small numbers.[2,3,13,15,37,39,41,43] More recently, there has been an increased detection of HPAI-positive seabirds or Charadriiformes[16,30] and increased mortality events in seabirds associated with HPAIV infection reported in the UK, Europe, and North America.[4,6,7,50] The data collected through the avian influenza (AI) wild bird passive surveillance scheme in Great Britain, whereby "found dead" wild birds are reported by the general public and submitted for testing, indicated a rise in HPAIV H5Nx positive birds within the *Laridae* family from 1.3% during the 2020-2021 season to 15% within the 2021-2022 season. Further, the number of HPAIV-positive Laridae birds, particularly the black-headed gull (*Chroicocephalus ridibundus*), in the UK and Europe remained high over spring-summer of 2023 with reports of significant mortality events at breeding colonies.[4]

One of the hypotheses for the enzootic transmission of HPAIV in wild birds in Europe is the maintenance in wild birds during summer in Northern Europe.[47] Previously, Anseriformes were thought to be responsible for virus transmission given potential virus adaptation in the host.[11,12] However, the expanded susceptibility of avian taxa to HPAIV and increased incidence of disease such as in the seabirds also challenges the status quo and introduces further uncertainty on the transmission dynamics at both the local and global levels.

The present report aims to investigate the gross and histological lesions and to evaluate the distribution of influenza A virus antigen in the tissues of seabirds in the Stercorariidae and Laridae families that died due to natural HPAIV H5N1 infection. The species examined include the great skua, long tailed skua (*Stercorarius longicaudus*), European herring gull, and black-headed gull.

Materials and methods

Post-mortem examination

Carcasses received at Scotland's Rural College, NatureScot, or Animal and Plant Health Agency (APHA) regional laboratories were frozen for transport and thawed for necropsy at APHA Weybridge (Supplemental Table S1). Post-mortem examinations were conducted by FZXL, NF, and AN in a microbiological safety cabinet within the high containment facility certified for level 4 specific-animal pathogen order. The herring gulls were obtained from wildlife rehabilitation centers (East Sussex and Cornwall) and were submitted for investigation as case reports. The black-headed gull carcasses were recovered from Birmingham, and the great skuas carcasses originated from colonies on Shetland, including Scatness (mainland), No Ness (mainland), Hermaness (island), Fair Isle (island), and Noss (island). The long tailed skua carcass originated from Clumlie (mainland). These specimens were collected as part of the UK AI wild bird passive surveillance scheme. Oropharyngeal and cloacal swabs and tissues were tested to confirm infection status with HPAIV H5N1 by standard tests, as described previously.[38] Major organs including pectoral sternal skin, pectoral skeletal muscle, heart, brain, spleen, kidney, nasal turbinate, trachea, lung, proventriculus, gizzard, liver, pancreas, duodenum, ovary, oviduct, and testis were fixed in 10% neutral-buffered formalin for microscopic evaluation.

Virological investigation

Oropharyngeal and cloacal swabs were collected from the great skuas, herring gulls, and black-headed gulls. Individual tissues were sampled from the great skuas and long tailed skua, while pooled tissues were collected from the herring gulls.

Oropharyngeal or cloacal swabs were placed into a microcentrifuge tube containing 1 ml of Leibovitz's L-15 Medium (ThermoFisher Scientific) with antibiotics. Each tube was gently mixed for a minimum of five seconds to elute the material from the swab before being allowed to stand at room temperature for a minimum of two minutes. Tissue suspensions (approximately 10% v/v) were prepared in phosphate-buffered saline containing antibiotics. Nucleic acid was extracted from each swab or tissue suspension sample by an automated process using the KingFisher Flex (ThermoFisher Scientific) with the reagents from the MagMAX CORE Nucleic Acid Purification Kit (ThermoFisher Scientific).

All nucleic acid extractions were tested by a suite of three AIV real-time reverse transcription polymerase chain reaction (RRT-PCR assays) for generic influenza A virus detection (*M* gene),[45] for specific detection of HPAIV *H5* AIV,[29] and by an *N1*-specific RRT-PCR to confirm the presence of H5N1 AIV according to the procedure described by Payungporn et al.[46] and adapted to the RRT-PCR chemistry at APHA by Slomka et al..[51] Samples producing a threshold cycle (Cq) value less than or equal to 36.0 were considered positive. All PCR amplifications were carried out in an AriaMx qPCR System (Agilent).

Histopathology and immunohistochemistry (IHC)

Formalin-fixed tissues were processed using routine histological methods into paraffin blocks. Tissues were sectioned at a 4 μm thickness and stained with haematoxylin and eosin for histological evaluation and immunohistochemical labelling using a mouse monoclonal IgG1 antibody against the nucleoprotein of influenza A virus (Statens Serum Institute, Denmark; HYB 340-05) at 1 in 4000 dilution for the detection of influenza viral antigen, as described previously.[42] The tissues were assessed on conventional light microscope for histopathology and scored as absent (-), minimal (+), mild (++), moderate (+++), or severe (++++). The abundance of virus antigens was scored as absent (-), rare (+), scattered (++), confluent (+++), or abundant (++++). Microscopic evaluations were performed by FZXL.[7] Detailed microscopic criteria are outlined in Supplemental Table S2 and S3.

Results

History and clinical findings

The captive herring gulls from two sites in England, East Sussex and Cornwall, each containing approximately 100 gulls, were reported to have exhibited clinical signs such as cyanotic heads, gasping, muscle twitching, diarrhea, and sudden deaths with mortality rates ranging between 20 and 50% (Supplemental Table S4). The carcasses of non-captive wild birds including great skuas, long tailed skuas, and black-headed gulls were retrieved after being found dead in the environment (Shetland and Birmingham). No clinical signs were recorded due to the lack of live observation.

Influenza virus detection

All birds tested positive for HPAIV H5N1 infection by RRT-PCR on oropharyngeal and cloacal swabs or tissues targeting *M*, HP *H5*, and *N1* genes. No viral RNA for avian avulavirus was detected using RRT-PCR for *L* gene. The oropharyngeal and cloacal swabs from great skuas yielded Cq values ranging from 20 to 28 and 22 to 33, respectively (Supplemental Table S1). Meanwhile, oropharyngeal and cloacal swabs from herring gulls showed Cq values ranging from 21 to 29 and 25 to 37, respectively. Black-headed gulls had Cq values ranging from 22 to 29 for oropharyngeal swabs and 19 to 31 for cloacal swabs. In cases where tissues were tested, the brain consistently yielded high viral loads with low Cq values ranging from 14 to 25 for great skuas and 15 to 20 for herring gulls (Supplemental Table S1 and S5). Other tissues including the trachea, lung, heart, liver, spleen, kidney, and intestine were positive on RRT-PCR. Where individual tissues were tested by RRT-PCR in the great skua, the respiratory tissues (trachea and lung) had lower Cq values compared to the intestines and kidneys (Supplemental Table S5). The oropharyngeal swabs also consistently yielded lower Cq values compared to the cloacal swabs. Similar trends were noted in the tissues and swabs from the herring gulls and the swabs from the black-headed gulls. Overall molecular virologic testing confirmed virus shedding from the oropharyngeal and cloacal orifices, with viral RNA being more abundant from the respiratory tissues and demonstrated multisystemic dissemination of HPAIV within the birds.

Post-mortem findings

All submitted birds were in fair to good body condition. On necropsy, the great skuas (n=8, 6 females and 2 males) were moderately autolysed. Only one of the birds had multifocal, approximately 1 to 2 mm in diameter, dark red ulcers on the gizzard close

to the proventricular-gizzard junction (Fig. 1a). The long tailed skua (n=1, male) was moderately autolysed and otherwise unremarkable. For the herring gulls (n=5, 2 male and 3 gender not determined) were mildly autolysed. Post-mortem examination findings included multifocal, faint tan patches within the pancreatic parenchyma (n=5; Fig. 1b), suggestive of necrosis; mild splenomegaly (n=3); and intestinal nematodiasis (n=1). Four black headed gulls (2 male and 2 female) were examined, but due to severe autolysis, the gross interpretation was limited.

Histopathology and viral immunohistochemistry

In the great skua, virus antigen was consistently detected in the heart, brain, kidney, lung, and pancreas of all birds examined (Table 1, Supplemental Table S6). The pancreas was particularly affected, with moderate to severe, multifocal to confluent areas of necrosis. These necrotic areas correlated with moderate to abundant distribution of virus antigens in all great skuas examined (Fig. 2a; n=8/8). Correlative viral IHC and histology also revealed viral-associated myocardial necrosis (Fig. 2b, n=2/8), splenic necrosis (Fig. 2c; n=3/4), and renal tubular necrosis (Fig. 2d; n=5/8), which were mild. In the gizzard of a great skua where ulceration was noted during the post-mortem examination (Fig. 1a), histological and IHC examinations confirmed of the presence of viral protein in the mucosa (Fig. 3a) and glandular epithelium (Fig. 3b). The mucosal damage was extensive and was replaced with necrotic cellular debris, degenerated heterophils, and fibrin deposition (Fig. 3a, b). Similar proventricular mucosa damage was also observed histologically in other two great skuas where lesions were not observed grossly. Nevertheless, viral immunolabelling in the proventriculus (n=6/8) and gizzard (n=5/8) were more common than histopathological changes (n=3/8, 1/8; respectively).

Further, virus antigens were detected in the ovaries (n=6/6) and oviducts (n=5/5) but not in the testis (n=0/2). In the ovaries, there was confluent distribution of viral antigens (Fig. 3c), mainly in the theca interna and occasionally transmurally in the pre-ovulatory follicles. Viral antigens were associated with necrosis within the tunica interna and blood vessels of the stroma, and the stromal wall was moderately to markedly expanded with lymphocytes, plasma cells, and fibrin deposits (Fig. 3d). In the oviduct, there was intra-luminal cellular debris, mucosal ulceration, and heterophilic and lymphocytic infiltration of submucosa wall. Viral antigens were present in the intra-luminal cellular debris (Fig. 3e), mucosa epithelium and submucosal cells (Fig. 3f).

Only one long tailed skua was examined, which had severe pancreatic necrosis, mild splenic and hepatic necrosis, and an abundance of viral antigens that were co-localised to these lesions (Table 1). Virus antigens were also detected in other organs where histopathological changes were absent, including the skin, skeletal muscle, heart, brain, kidney, trachea, gizzard, and testis.

In the herring gulls, viral antigens were found in the pancreas (n=5/5, Fig. 4a), spleen (n=5/5, Fig, 4b), brain (n=5/5, Fig, 4c), and lung (n=5/5), and were consistently associated with histological lesions. Pancreatic necrosis was multifocal to confluent and with a range of minimal to severe changes (Fig. 4a). In the spleen, mild lymphoid depletion was associated with lymphoid necrosis (Fig. 4b). In the brain, there was mild neuronal necrosis and dispersed degenerated heterophils within the neuropil. In the cerebellum, there was occasional loss of Purkinje cells attributed to viral infection (Fig. 4c). Rhinitis ranged from mild changes including scant heterophilic exudate with occasional intra-epithelial heterophils, or in severe cases with abundant exudation, complete loss of mucosa with submucosa necrosis and fibrin deposition (Fig. 4d). In the lungs, there were mild to moderate air capillary necrosis (Fig. 4e) with occasional

fibrin deposition in air capillary walls. A gizzard lesion was detected in one herring gull and was characterized by necrosis of the mucosa-submucosa and loss of koilin with virus antigens detected in the mucosa epithelium and lymphoid cells within the submucosa (Fig. 4f). Incidental findings included the presence of an intestinal cestode and proventricular nematodes in two herring gulls but were not associated with overt intestinal lesions.

Four black-headed gulls were examined microscopically, but the histological interpretation was hindered by the state of autolysis. Within the limits of tissue preservation, moderate air capillary necrosis was observed, and organs such as the brain, kidney, and liver exhibited only minimal mononuclear inflammation. On the other hand, viral antigens were abundant in the brain, heart, and lung (Table 1). Ovary was sampled from one black-headed gull, and immunohistochemical labeling revealed rare viral antigens within the blood vessels of the ovarian stroma, but no overt histological changes were detected.

Discussion

During the 2020-2021 and 2021-2022 outbreaks of HPAIV in the UK, there has been an increased detection of HPAIV H5N1 in seabirds of the Stercorariidae and Laridae families.[5,7,16] This investigation of naturally acquired HPAIV infection revealed that gross lesions were limited to pancreatic necrosis in the herring gull and gizzard ulceration in the great skua. The pancreatic changes were less conspicuous compared to those in Galliformes and Anseriformes and required immunohistochemical confirmation. Microscopic evaluation confirmed a multi-systemic HPAIV infection including neuro-, pneumo-, lymphoid-, and cardiomyotropism, which was likely contributary to the mortalities seen. In addition, acute reproductive damage in female

great skua was noted. Overall, skua and gull birds are highly susceptible to developing lesions in multiple organ systems following HPAIV infection.

The most common and severe lesion in all birds examined was pancreatic necrosis associated with viral infection (except in the black-headed gulls where the pancreas was unavailable), followed by splenic necrosis and pneumonia (except in the long tailed skua). Such lesions are similar to those reported from experimentally challenged common gulls (*Larus canus*), black-headed gulls, and herring gulls with pre-clade 2.3.4.4.b and 2.3.4.4b isolates of HPAIV H5N1,[10,24,49,53] as well as report of naturally infected sandwich terns (*Thalasseus sandvicensis*) with the contemporaneous H5N1.[49,50] The multisystemic infection and lesions observed in skua and gull birds, including vascular tropism, are similar to those typically seen in HPAIV-infected Galliformes poultry,[20,40] emphasizing the susceptibility of skua and gull birds to the contemporaneously circulating HPAIV 2.3.4.4b H5N1.

Avian influenza viruses are known for the preferential binding to the α-2,3 sialic acid residues.[14] Based on lectin histochemistry on other gull species including American herring gulls (*Larus smithsonianus*), laughing gulls (*Leucophaeus atricilla*), and ring-billed gulls (*Larus delawarensis*), the respiratory epithelium commonly express both α-2,3 and α-2,6 sialic acids, whereas the intestinal tracts express predominantly α-2,3 and rarely α-2,6 sialic acids.[19] Historically, gull species from the Order Laridae have been associated with infection with LPAIV including H11, H13, and H16 subtypes.[25,35,57] These infections have been primarily associated with replication in epithelial cells of intestine and thus faecal-oral transmission of LPAIV in the black headed gulls has been proposed.[26] In the case of HPAIV, natural infection has been reported in great skuas, European herring gulls, black-headed gulls, and great black-backed gulls (*Larus marinus*).[2,3,7,13,15,37,41,43] Intestinal infections have rarely been

observed with experimental studies using H5N1 clade 2.2 viruses in common gulls[24] and laughing gulls infected with an 'Eurasian-lineage' of H5N1.[9] However, the cellular tropism of HPAIV has not been reviewed in detail in the previous studies, hence limiting the understanding of the pathobiology.

The viral tropism of the enteric or respiratory system has significant implications for the dynamics of disease transmission. In this report, viral immunolabeling in the trachea, proventriculus, gizzard, and duodenum of the great skua were more frequently detected than those great skua from the previous epizootic event in Great Britain in 2021.[7] The immunolabeling of the glandular epithelium of the gizzard in particular further confirms virus-specific immunolabeling of epithelial cells within the digestive tract. In addition, similar respiratory and enterotropism of HPAIV was observed in the long tailed skua, herring gull, and black-headed gull in this study. Importantly, viral RNA was more abundant in the oropharyngeal compared to the cloacal swabs, with similar differences observed between respiratory and enteric tissues, which could be related to the expression pattern of α-2,3 and α-2,6 sialic acids in the respiratory and intestinal epithelium.[19] Contrary to the proposed enteric tropism and adaptation of Gs/Gd HPAIV in wild waterbirds,[11,12] the dual tropism for the respiratory and enteric systems suggests that the contemporary HPAI 2.3.4.4b viruses are highly permissive within the skua and gull birds.

The pathway of incursion in free-ranging seabirds is not understood but has been proposed to be either independent incursion or onward introductions from species movements between colonies and the movement of seabirds between mainland and islands particularly during the breeding season.[16,50] Herring gulls and great skua can opportunistically predate or scavenge on other birds,[16,28,34,58] and this was observed in the outbreak in gannet colonies. Further, contact transmission between common

gulls and European herring gulls have been documented previously in experimental infections with HPAIV H5N1 clade 2.2 and H5N8 clade 2.3.4.4b.[24,53] More recent HPAIV H5N1 outbreaks (June and August 2022) in wild bird rescue centers / hospitals in England (East Sussex and Cornwall) have been confirmed in herring gulls. After epidemiological assessment, the most likely source of infection appeared to be the introduction of infected / diseased herring gulls within the premises, which had then transmitted the disease to the resident gulls of the same species within and among enclosures (Animal and Plant Health Agency, unpublished data). During the breeding season, skuas and gulls often gather in large numbers to nest, feed, and bath, which increases the likelihood of close contact and potential transmission of HPAIV, particularly if virus replication occurs in the respiratory and intestinal tracts.[16] Infections through such contact can lead to birds from other colonies becoming exposed and infected, who can then disseminate the virus to new localities and susceptible avian species. The HPAI outbreak in the great skua colony in the UK has resulted in approximately 10% mortality during the summer of 2022, and outbreaks in captive herring gulls reported in this study have had mortality rates ranging from 20% to 50%. Although not examined in this study, subsets of skua or gull birds that develop mild or subclinical disease could potentially facilitate transmission over larger areas.

The distribution and ecology of seabird populations also challenge the current understanding of HPAIV transmission at a global level. Both long tailed skua and great skua are transitory migrant birds. Long tailed skuas are a passage migrant in the UK and breed in Arctic region,[23] whereas great skuas migrate to the northernmost isles of the UK in summer for breeding and return to the coasts of Spain and Africa, and as far as Brazilian and Argentinian coasts for wintering.[27,44] This is in contrast to the black-headed gulls that are found across the UK,[31] and herring gulls are found throughout

the year around the UK coastline and inland around rubbish tips, fields, large reservoirs, and lakes, especially during the winter months.[33] Recent ring-recovery data revealed that great skua, European herring gulls, and black-headed gulls migrate between Europe to Iceland and other North Atlantic islands, and to North America.[13] The pelagic and migratory nature of gulls have led to the suggestion of intercontinental dissemination and shaping of influenza A virus evolution.[22,25,48,55,59] Further, these seabirds are often found in areas with high seal populations plus other scavenging mammals that can predate on sick or dead birds, and result in exposures of other host populations to infectious materials either directly or indirectly through the environment.[17]

Apart from the increased mortality in seabirds during 2022, which has resulted in an immediate impact upon populations, there is a significant deficit in knowledge on the impact of infectious diseases on population structures across these species. However, a trend towards a reduction in breeding abundance in the UK for herring gulls, black-headed gulls, and great skuas has been noted.[31-33] The pathogenic mechanism of HPAIV on reproductive organs of wild bird is poorly documented. Previous reports have demonstrated epithelial labelling of viral antigen in the oviduct of common buzzards and peregrine falcons infected with HPAIV.[56] In domestic poultry, both HPAIV and LPAIV infections can lead to short to long-term reductions in egg production or embryonic death because of viral-induced lesions in the ovaries, oviduct, or conceptus.[8,36,52,54] There has been an increased detection of reproductive diseases in laying poultry, both Galliformes and Anseriformes, during the 2022 epizootic season in the UK, which can be attributed to virus infections (Lean F, unpublished). However, the impact on the layer poultry sector, where an abundance of eggs is produced daily, cannot be compared to seasonal reproductive cycle in seabirds and as such the

longer-term impact on population densities for these species will require monitoring to assess population recovery.

In this study, great skuas and black-headed gulls were frozen and thawed for necropsy, which can introduce artifacts that could affect histopathological interpretation and the antigenicity of virus proteins for IHC. Additionally, the small sample size for long tailed skua and black-headed gull, and the gender skew (predominantly female great skuas and male gull birds) may limit meaningful comparison. It is important to note that the availability of carcasses received from wild bird surveillance is dependent on natural events. The age of birds could also potentially have an impact on disease outcome. It was documented from the two outbreaks from captive settings that only the younger herring gulls, but not the older birds, were clinically affected. It would have been useful to sample birds of different age groups and disease states, but such work has not been possible due to resource constraints during multiple disease outbreaks. Finally, this study only evaluated a subset of species within Stercorariidae and Laridae. Other seabird family such as Alcidae, Pelecanidae, Phalacrocoracidae, and Procellariidae have tested positive for HPAIV 2.3.4.4b during the epizootic;[1] therefore, further study is warranted to understand the susceptibility of other seabird species and the associated lesions with HPAIV infection.

In conclusion, this study demonstrates the susceptibility and pathology of a subset of Laridae and Suliformes following naturally acquired infection with HPAIV H5N1 clade 2.3.4.4b. The rapid mortality is associated with multisystemic dissemination of virus and resultant tissue damage. Epitheliotropism in the respiratory and digestive systems enables shedding of virus and facilitates transmission. Reproductive pathology is also

noted amongst the female great skua, but the longer-term impact on population fecundity warrants further investigation.

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Conflict of interest statement

The authors declare no conflict of interests.

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Ethical statement

No ethical approval was required as carcass and tissue were derived from diagnostic investigations.

Author contribution statement

F.Z.X.L., M.F., N.F., G.T., C.R., P.H., C.M. involved in conceptualisation of the investigations. F.Z.X.L., N.F. performed the necropsies. F.Z.X.L. conducted formal analysis. A.N., A.C.B., S.M.R., I.H.B., C.M. provided project leadership, financial, and laboratory resources. F.Z.X.L. wrote the original draft. All authors reviewed and edited the manuscript.

References

1 . Adlhoch C, Fusaro A, Gonzales JL, et al. Avian influenza overview September - December 2022. *EFSA journal European Food Safety Authority*. 2023; **21:** e07786.

2 . Adlhoch C, Fusaro A, Gonzales JL, et al. Avian influenza overview February - May 2021. *EFSA journal European Food Safety Authority*. 2021; **19:** e06951.

3 . Adlhoch C, Fusaro A, Gonzales JL, et al. Avian influenza overview June - September 2022. *EFSA journal European Food Safety Authority*. 2022; **20:** e07597.

4 . Adlhoch C, Fusaro A, Gonzales JL, et al. Avian influenza overview March - April 2023. *EFSA journal European Food Safety Authority*. 2023; **21:** e08039.

5 . APHA: Updated outbreak assessment #28 highly pathogenic avian influenza (HPAI) in the UK and Europe. 2022

6 . APHIS: 2022 detections of highly pathogenic avian influenza in wild birds. 2022

7 . Banyard AC, Lean FZX, Robinson C, et al. Detection of highly pathogenic avian influenza virus H5N1 clade 2.3.4.4b in great skuas: A species of conservation concern in Great Britain. *Viruses*. 2022; **14**.

8 . Bonfante F, Mazzetto E, Zanardello C, et al. A G1-lineage H9N2 virus with oviduct tropism causes chronic pathological changes in the infundibulum and a long-lasting drop in egg production. *Vet Res*. 2018; **49:** 83.

9 . Brown JD, Stallknecht DE, Beck JR, Suarez DL, Swayne DE. Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. *Emerg Infect Dis*. 2006; **12:** 1663-1670.

10 . Brown JD, Stallknecht DE, Swayne DE. Experimental infections of herring gulls (Larus argentatus) with H5N1 highly pathogenic avian influenza viruses by intranasal inoculation of virus and ingestion of virus-infected chicken meat. *Avian pathology : journal of the WVPA*. 2008; **37:** 393-397.

11 . Caliendo V, Leijten L, Begeman L, et al. Enterotropism of highly pathogenic avian influenza virus H5N8 from the 2016/2017 epidemic in some wild bird species. *Veterinary Research*. 2020; **51:** 117.

12 . Caliendo V, Leijten L, van de Bildt M, et al. Tropism of highly pathogenic avian influenza H5 viruses from the 2020/2021 epizootic in wild ducks and geese. *Viruses*. 2022; **14:** 280.

13 . Caliendo V, Lewis NS, Pohlmann A, et al. Transatlantic spread of highly pathogenic avian influenza H5N1 by wild birds from Europe to North America in 2021. *Sci Rep*. 2022; **12:** 11729.

14 . de Graaf M, Fouchier RAM. Role of receptor binding specificity in influenza A virus transmission and pathogenesis. *The EMBO Journal*. 2014; **33:** 823-841.

15 . Ellis TM, Bousfield RB, Bissett LA, et al. Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. *Avian pathology : journal of the WVPA*. 2004; **33:** 492-505.

16 . Falchieri M, Reid SM, Ross CS, et al. Shift in HPAI infection dynamics causes significant losses in seabird populations across Great Britain. *Vet Rec*. 2022; **191:** 294-296.

17 . Floyd T, Banyard AC, Lean FZX, et al. Encephalitis and death in wild mammals at a rehabilitation center after infection with highly pathogenic avian influenza A(H5N8) virus, United Kingdom. *Emerg Infect Dis*. 2021; **27:** 2856-2863.

18 . Fouchier RA, Munster VJ. Epidemiology of low pathogenic avian influenza viruses in wild birds. *Rev Sci Tech*. 2009; **28:** 49-58.

19 . França M, Stallknecht DE, Howerth EW. Expression and distribution of sialic acid influenza virus receptors in wild birds. *Avian pathology : journal of the WVPA*. 2013; **42:** 60-71.

20 . Gaide N, Lucas MN, Delpont M, et al. Pathobiology of highly pathogenic H5 avian influenza viruses in naturally infected Galliformes and Anseriformes in France during winter 2015-2016. *Vet Res*. 2022; **53:** 11.

21 . Gambaryan AS, Matrosovich TY, Boravleva EY, et al. Receptor-binding properties of influenza viruses isolated from gulls. *Virology*. 2018; **522:** 37-45.

460 22 . Gass Jr JD, Dusek RJ, Hall JS, et al. Global dissemination of influenza A virus is
461 driven by wild bird migration through arctic and subarctic zones. *Molecular ecology*.
462 **n/a**.

463 23 . Gilg O, Moe B, Hanssen SA, et al. Trans-equatorial migration routes, staging sites
464 and wintering areas of a high-arctic avian predator: the long-tailed skua (Stercorarius
465 longicaudus). *PLOS ONE*. 2013; **8:** e64614.

466 24 . Gulyaeva MA, Sharshov KA, Zaykovskaia AV, Shestopalova LV, Shestopalov AM.
467 Experimental infection and pathology of clade 2.2 H5N1 virus in gulls. *Journal of*
468 *veterinary science*. 2016; **17:** 179-188.

469 25 . Hill NJ, Bishop MA, Trovão NS, et al. Ecological divergence of wild birds drives
470 avian influenza spillover and global spread. *PLOS Pathogens*. 2022; **18:** e1010062.

471 26 . Höfle U, Van de Bildt MW, Leijten LM, et al. Tissue tropism and pathology of
472 natural influenza virus infection in black-headed gulls (Chroicocephalus ridibundus).
473 *Avian pathology : journal of the WVPA*. 2012; **41:** 547-553.

474 27 . Hoyo Jd, Elliott A, Sargatal J: Handbook of the birds of the world – hoatzin to auks,
475 vol. 3, Lynx Edicions, Barcelona, Spain, 1996

476 28 . Ingraham LR, Oswald SA, Son E, Arnold JM. Opportunistic predation of birds by
477 breeding Herring Gulls (Larus argentatus). *PLoS One*. 2020; **15:** e0239180.

29 . James J, Seekings AH, Skinner P, et al. Rapid and sensitive detection of high pathogenicity Eurasian clade 2.3.4.4b avian influenza viruses in wild birds and poultry. *J Virol Methods*. 2022; **301:** 114454.

30 . Jimenez-Bluhm P, Siegers JY, Tan S, et al. Detection and phylogenetic analysis of highly pathogenic A/H5N1 avian influenza clade 2.3.4.4b virus in Chile, 2022. *Emerg Microbes Infect*. 2023**:** 2023.2002.2001.526205.

31 . JNCC: Black-headed gull (Chroicocephalus ridibundus). 2021

32 . JNCC: Great skua (Stercorarius skua). 2021

33 . JNCC: Herring gull (Larus argentatus). 2021

34 . Jones T, Smith C, Williams E, Ramsay A. Breeding performance and diet of Great Skuas Stercorarius skua and Parasitic Jaegers (Arctic Skuas) S. parasiticuson the west coast of Scotland. *Bird Study*. 2010; **55:** 257-266.

35 . Karamendin K, Kydyrmanov A, Zhumatov K, et al. Phylogenetic analysis of avian influenza viruses of H11 subtype isolated in Kazakhstan. *Virus Genes*. 2011; **43:** 46-54.

36 . Kinde H, Read DH, Daft BM, et al. The occurrence of avian influenza A subtype H6N2 in commercial layer flocks in Southern California (2000-02): clinicopathologic findings. *Avian Dis*. 2003; **47:** 1214-1218.

37 . Kleyheeg E, Slaterus R, Bodewes R, et al. Deaths among wild birds during highly pathogenic avian influenza A(H5N8) virus outbreak, the Netherlands. *Emerg Infect Dis*. 2017; **23:** 2050-2054.

38 . Lean FZX, Vitores AG, Reid SM, 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.

39 . Li M, Liu H, Bi Y, et al. Highly pathogenic avian influenza A(H5N8) virus in wild migratory birds, Qinghai Lake, China. *Emerg Infect Dis*. 2017; **23:** 637-641.

40 . Liang Y, Hjulsager CK, Seekings AH, et al. Pathogenesis and infection dynamics of high pathogenicity avian influenza virus (HPAIV) H5N6 (clade 2.3.4.4b) in pheasants and onward transmission to chickens. *Virology*. 2022; **577:** 138-148.

41 . Liu J, Xiao H, Lei F, et al. Highly pathogenic H5N1 influenza virus infection in migratory birds. *Science*. 2005; **309:** 1206.

42 . Löndt BZ, Nunez A, Banks J, et al. Pathogenesis of highly pathogenic avian influenza A/turkey/Turkey/1/2005 H5N1 in Pekin ducks (Anas platyrhynchos) infected experimentally. *Avian pathology : journal of the WVPA*. 2008; **37:** 619-627.

43 . Madslien K, Moldal T, Gjerset B, et al. First detection of highly pathogenic avian influenza virus in Norway. *BMC Vet Res*. 2021; **17:** 218.

514 44 . Magnusdottir E, Leat EHK, Bourgeon S, et al. Wintering areas of great skuas
515 Stercorarius skua breeding in Scotland, Iceland and Norway. *Bird Study*. 2012; **59:** 1-
516 9.

517 45 . Nagy A, Černíková L, Kunteová K, et al. A universal RT-qPCR assay for "One
518 Health" detection of influenza A viruses. *PLoS One*. 2021; **16:** e0244669.

519 46 . Payungporn S, Chutinimitkul S, Chaisingh A, et al. Single step multiplex real-time
520 RT-PCR for H5N1 influenza A virus detection. *J Virol Methods*. 2006; **131:** 143-147.

521 47 . Pohlmann A, King J, Fusaro A, et al. Has epizootic become enzootic? Evidence
522 for a fundamental change in the infection dynamics of highly pathogenic avian
523 influenza in Europe, 2021. *mBio*. 2022; **13:** e0060922.

524 48 . Postnikova Y, Treshchalina A, Boravleva E, et al. Diversity and reassortment rate
525 of influenza A viruses in wild ducks and gulls. *Viruses*. 2021; **13**.

526 49 . Ramis A, van Amerongen G, van de Bildt M, et al. Experimental infection of highly
527 pathogenic avian influenza virus H5N1 in black-headed gulls (Chroicocephalus
528 ridibundus). *Vet Res*. 2014; **45:** 84.

529 50 . Rijks JM, Leopold MF, Kühn S, et al. Mass mortality caused by highly pathogenic
530 influenza A(H5N1) virus in sandwich terns, the Netherlands, 2022. *Emerging*
531 *Infectious Diseases*. 2022; **28:** 2538-2542.

532 51 . Slomka MJ, To TL, Tong HH, et al. Challenges for accurate and prompt molecular
533 diagnosis of clades of highly pathogenic avian influenza H5N1 viruses emerging in
534 Vietnam. *Avian pathology : journal of the WVPA*. 2012; **41:** 177-193.

535 52 . Stephens CB, Spackman E, Pantin-Jackwood MJ. Effects of an H7 highly
536 pathogenic and related low pathogenic avian influenza virus on chicken egg
537 production, viability, and virus contamination of egg contents and surfaces. *Avian Dis*.
538 2020; **64:** 143-148.

539 53 . Tarasiuk K, Kycko A, Knitter M, et al. Pathogenicity of highly pathogenic avian
540 influenza H5N8 subtype for herring gulls (Larus argentatus): impact of homo- and
541 heterosubtypic immunity on the outcome of infection. *Veterinary Research*. 2022; **53:**
542 108.

543 54 . Uchida Y, Takemae N, Tanikawa T, Kanehira K, Saito T. Transmission of an
544 H5N8-subtype highly pathogenic avian influenza virus from infected hens to laid eggs.
545 *Avian Dis*. 2016; **60:** 450-453.

546 55 . Van Borm S, Rosseel T, Vangeluwe D, et al. Phylogeographic analysis of avian
547 influenza viruses isolated from Charadriiformes in Belgium confirms intercontinental
548 reassortment in gulls. *Arch Virol*. 2012; **157:** 1509-1522.

549 56 . van den Brand JM, Krone O, Wolf PU, et al. Host-specific exposure and fatal
550 neurologic disease in wild raptors from highly pathogenic avian influenza virus H5N1
551 during the 2006 outbreak in Germany. *Vet Res*. 2015; **46:** 24.

552 57 . Verhagen JH, Majoor F, Lexmond P, et al. Epidemiology of influenza A virus
553 among black-headed gulls, the Netherlands, 2006-2010. *Emerg Infect Dis*. 2014; **20:**
554 138-141.

555 58 . Votier SC, Bearhop S, Crane JE, Manuel Arcos J, Furness RW. Seabird predation
556 by great skuas Stercorarius skua– intra-specific competition for food? *Journal of Avian*
557 *Biology*. 2007; **38:** 234-246.

558 59 . Wille M, Robertson GJ, Whitney H, et al. Extensive geographic mosaicism in avian
559 influenza viruses from gulls in the northern hemisphere. *PLoS One*. 2011; **6:** e20664.

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Figure Legends

Figure 1. Gross lesions of highly pathogenic avian influenza virus H5N1 infected seabirds. (a) Gizzard, great skua (*Stercorarius skua*). Multifocal dark red depressions (white arrow) are present on the mucosa of the gizzard close to the proventricular-gizzard junction. (b) Pancreas, European herring gull (*Larus argentatus*). Multifocal to coalescing pale tan areas in the pancreas are consistent with necrosis (arrowheads).

Figure 2. Microscopic findings of great skua (*Stercorarius skua*) infected with highly pathogenic avian influenza virus H5N1. Hematoxylin and eosin. (a) Pancreas. Severe, confluent, pancreatic necrosis. (b) Heart. Moderate, multifocal, myocardial necrosis. (c) Spleen. Mild, multifocal, splenic necrosis. (d) Kidney. Mild, multifocal, renal tubular necrosis. Arrows indicate area of necrosis. Insets (a-d): Co-localization of viral antigens with areas of necrosis. Influenza A nucleoprotein immunohistochemistry.

Figure 3. Microscopic findings of great skua (*Stercorarius skua*) infected with highly pathogenic avian influenza virus H5N1. Hematoxylin and eosin. (a, b) Gizzard. Moderate, focal, gizzard necrosis and fracturing of koilin (b, arrowhead), with evidence of mucosa epithelial degeneration (b, arrow), and deposition of cellular debris, degenerated heterophils, extravasated erythrocytes, and fibrin within disrupted koilin layer. (c-d) Ovary. (c) Moderate, diffuse, oophoritis. (d) The theca interna and stroma are necrotised (arrow), and there is fibrin deposition within the mural typically around blood vessels (arrowhead). (e-f) Oviduct. (e) Moderate necrotizing salpingitis, with abundant intra-luminal debris. (f) The mucosa is eroded and infiltrated with heterophils and

lymphocytes. Insets (a-f): Co-localization of viral antigens within areas of necrosis and intra-luminal debris. Influenza A nucleoprotein immunohistochemistry.

Figure 4. Microscopic findings of European herring gull (*Larus argentatus*) infected with highly pathogenic avian influenza virus H5N1. Hematoxylin and eosin. (a) Pancreas. Mild, multifocal, necrosis of the pancreatic acinar cells characterized by marked cytoplasmic swelling (arrowhead) and nuclear pyknosis. (b) Spleen. Mild, multifocal necrosis (arrow) of splenic white pulp. (c) Cerebellum. Minimal, multifocal, neuronal necrosis with scattered heterophilic infiltration of neuropil (arrow) and loss of Purkinje cells (arrowhead). (d) Nasal cavity. Severe, confluent, necrotizing rhinitis with extensive loss or attenuation of epithelial cells (arrow) and fibrin deposition within the submucosa (arrowhead). (e) Lung. Moderate, multifocal, air capillary necrosis characterized by expansion of the interstitium with karyorrhectic debris (arrowhead), lymphohistiocytic infiltration, and hemorrhage. (f) Gizzard. Mild, multifocal, gizzard necrosis with disruption of the mucosa-submucosa, accumulation of karyorrhectic debris and fibrin within the submucosa (arrowhead), and loss of koilin. Insets (a-f): Co-localization of viral antigens with areas of necrosis. Influenza A nucleoprotein immunohistochemistry.

Table 1. Summary of histopathology and viral immunohistochemistry (IHC) findings.

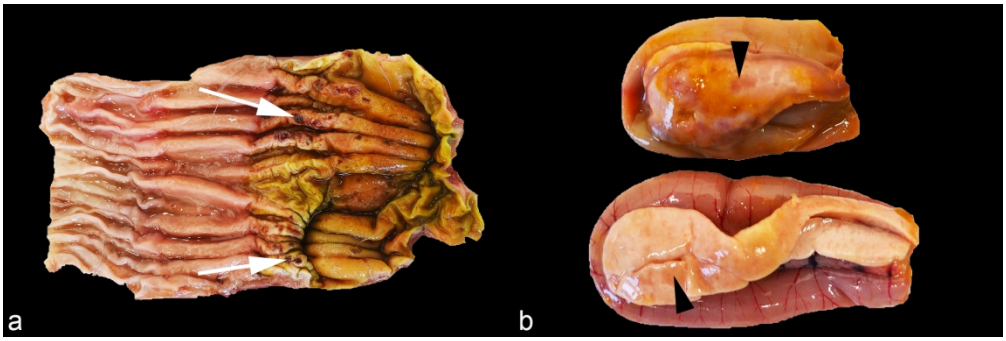
Tissue	Great Skua (n=8) *Stercorarius skua*				Long tailed skua (n=1) *Stercorarius longicaudus*				European herring gull (n=5) *Larus argentatus*				Black-headed gull (n=4) *Chroicocephalus ridibundus*			
	Histopathology		IHC		Histopathology		IHC		Histopathology		IHC		Histopathology		IHC	
	n (%)	Grade [a, c]	n (%)	Grade [b, c]	n (%)	Grade	n (%)	Grade	n (%)	Grade	n (%)	Grade	n (%)	Grade	n (%)	Grade
Skin	0/8 (0)	-	5/8 (63)	+	0/1 (0)	-	1/1 (100)	+	0/4 (0)	-	0/4 (0)	-	0/4 (0)	-	1/4 (25)	+
Skeletal muscle	0/8 (0)	-	7/8 (88)	+	0/1 (0)	-	1/1 (100)	++	0/5 (0)	-	5/5 (100)	+	1/3 (33)	+	3/3 (100)	+ to ++
Heart	2/8 (25)	+	8/8 (100)	+ to +++	0/1 (0)	-	1/1 (100)	++++	1/5 (20)	++	5/5 (100)	+ to ++	0/4 (0)	-	4/4 (100)	+ to ++++
Brain	3/8 (38)	+ to ++	8/8 (100)	+ to ++++	0/1 (0)	-	1/1 (100)	+++	4/5 (80)	++	5/5 (100)	++ to ++++	1/4 (25)	+	4/4 (100)	++ to ++++
Spleen	3/4 (75)	++	3/4 (75)	++ to ++++	1/1 (100)	++	1/1 (100)	++++	4/5 (80)	+	5/5 (100)	+ to ++	n/a	n/a	n/a	n/a
Kidney	5/8 (63)	+	8/8 (100)	+ to ++	0/1 (0)	-	1/1 (100)	++++	0/5 (0)	-	3/5 (60)	+	1/1 (100)	+	1/1 (100)	++
Nasal turbinate	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	4/4 (100)	+ to ++++	3/4 (75)	++ to +++	n/a	n/a	n/a	n/a
Trachea	0/8 (0)	-	5/8 (63)	1 to ++	0/1 (0)	-	1/1 (100)	++	3/3 (100)	+ to ++	3/3 (100)	+	n/a	n/a	n/a	n/a
Lung	1/8 (13)	+	8/8 (100)	+ to ++++	1/1 (100)	+	1/1 (100)	++++	3/5 (60)	+	5/5 (100)	+ to ++++	1/2 (50)	+++	2/2 (100)	++++
Proventriculus	3/8 (38)	+ to +++	6/8 (75)	+ to ++	n/a	n/a	n/a	n/a	0/5 (0)	-	1/5 (20)	+	0/4 (0)	-	2/4 (50)	+ to ++
Gizzard	1/8 (13)	+++	5/8 (63)	+ to ++	0/1 (0)	++	1/1 (100)	++	1/5 (20)	+	1/5 (20)	++	0/3 (0)	-	3/3 (100)	+
Liver	2/8 (25)	+ to ++	7/8 (88)	+ to ++	1/1 (100)	+	1/1 (100)	++++	3/5 (60)	+ to ++	4/5 (80)	+ to ++	1/3 (33)	+	1/3 (33)	++
Pancreas	8/8 (100)	+++ to ++++	8/8 (100)	+++ to ++++	1/1 (100)	++++	1/1 (100)	+++	5/5 (100)	+ to ++++	5/5 (100)	++ to ++++	n/a	n/a	n/a	n/a
Duodenum	1/8 (13)	+	5/8 (63)	+ to ++	n/a	n/a	n/a	n/a	2/5 (40)	+	3/5 (60)	+	n/a	n/a	n/a	n/a
Ovary	6/6 (100)	+ to +++	6/6 (100)	+ to ++++	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0/1 (0)	-	1/1 (100)	+
Oviduct	5/5 (100)	+ to +++	5/5 (100)	++ to +++	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Testis	0/2 (0)	-	0/2 (0)	-	0/1 (0)	-	1/1 (100)	++	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

[a] Histopathology grade: Absent -, minimal +, mild ++, moderate +++, severe ++++

[b] Immunohistochemical grade: Absent -, rare +, scattered ++, confluent +++, abundant ++++

[c] The provided grades represent the range of lesion severity and abundance of viral antigen

n/a – tissue not available for assessment



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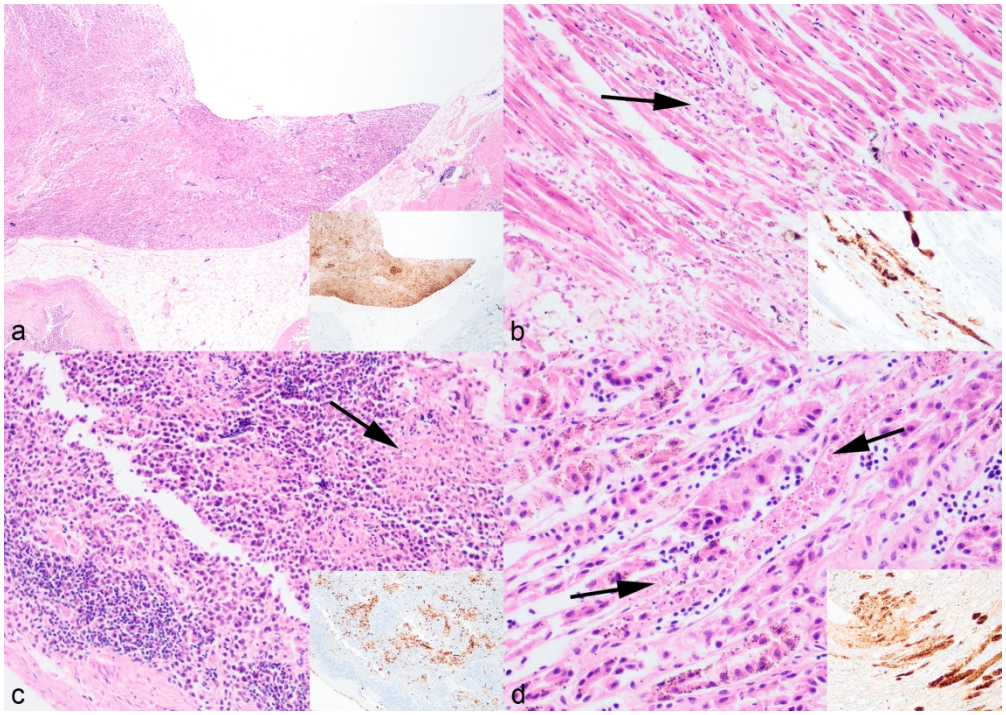

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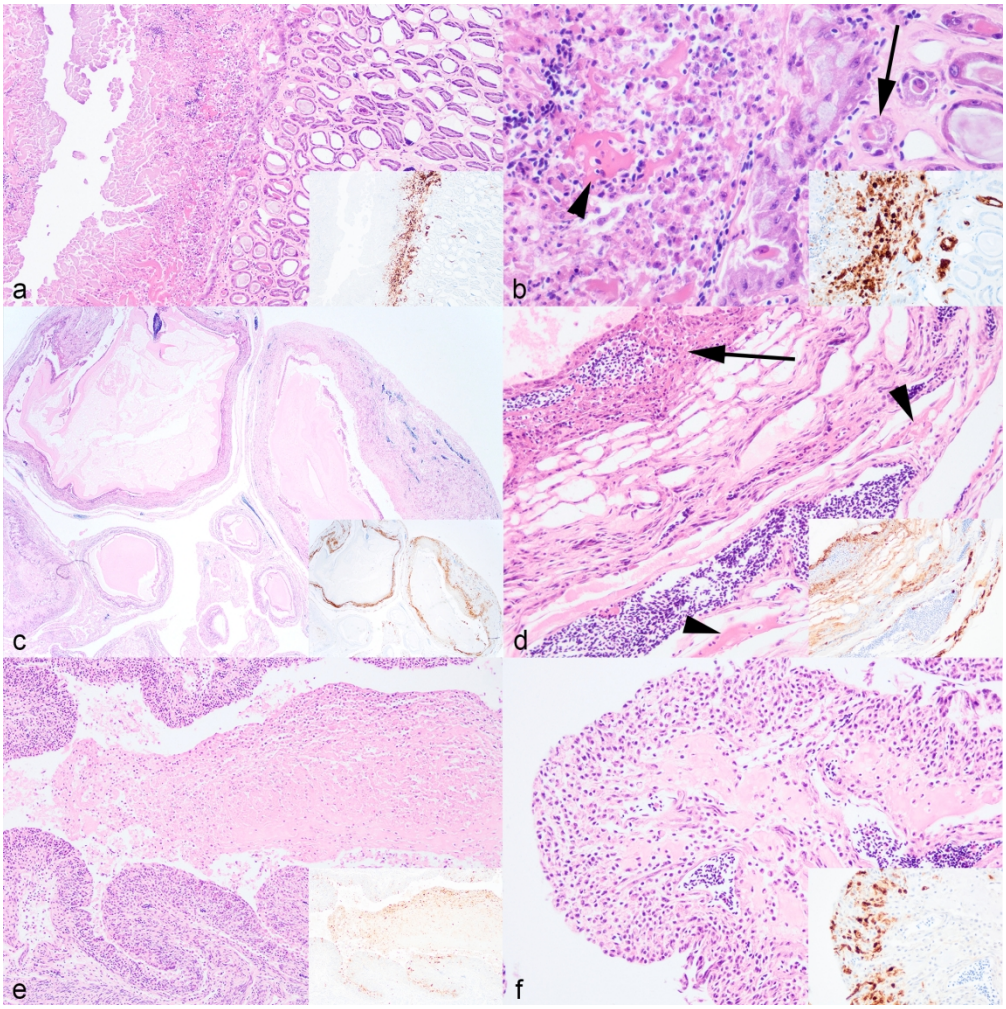

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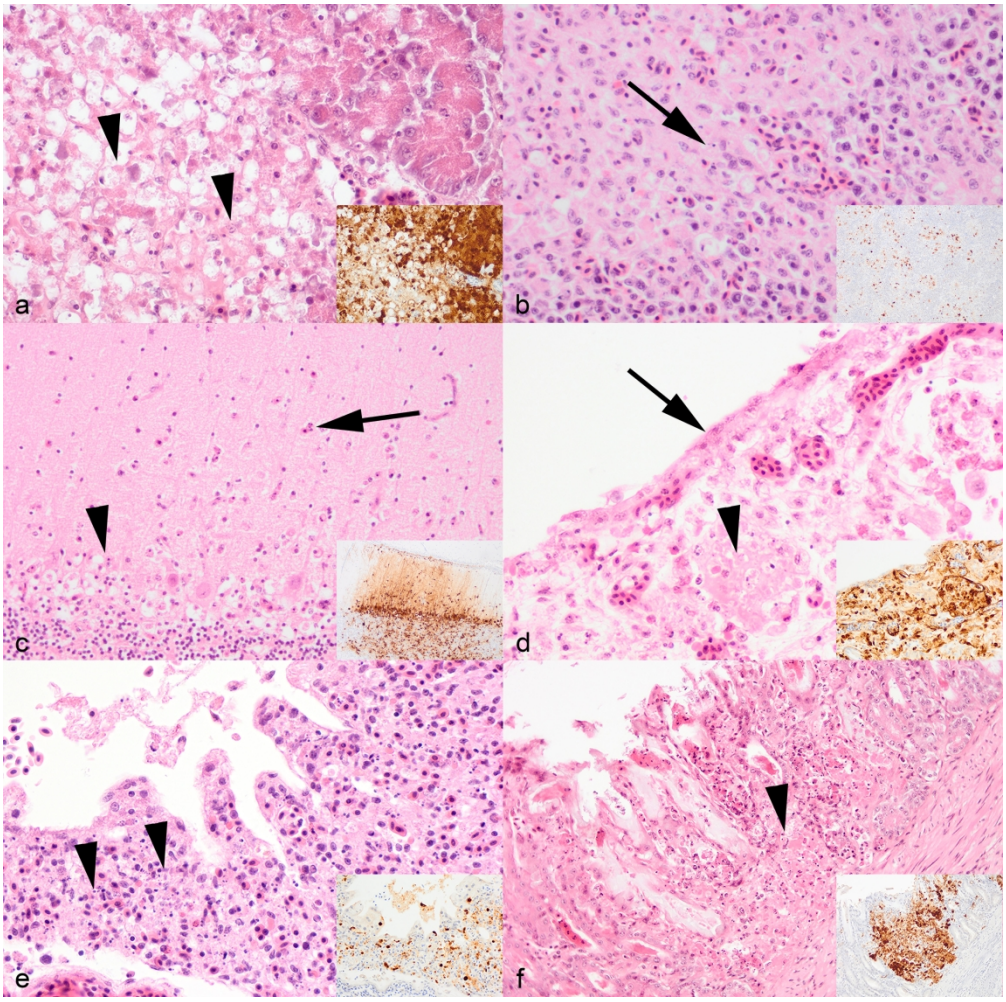

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Supplemental Table S1. Description of cases, degree of autolysis, body condition, gross findings, and virology results.

Necropsy Date	Carcass Retrieved Date	Species	ID	Location	Gender	Fresh/ frozen	Degree of autolysis	Body condition	Gross findings	Virology Results (Oropharyngeal OP/Cloacal CP swab)	Virology Results (Tissue)
5/16/2022	4/27/2022	Great skua	Bird 1	Scatness	Female	Frozen	Moderate	Good	Crop, proventriculus and gizzard are empty. Scant digestive or fecal material in the intestine and colon. On the mucosa surface of proximal gizzard are multifocal dark red, depressed (ulcer) areas, measuring 2 to 4 mm diameter	OP: 28.10 (M-gene), 25.46 (HP H5), 28.00 (N1) CP: 32.67 (M-gene), 27.33 (HP H5), 29.75 (N1)	All positive (see tissue PCR tab)
	5/7/2022		Bird 2	Noss	Female	Frozen	Moderate	Good	Crop, proventriculus and gizzard are empty. Scant digestive or fecal material in the intestine and colon. At the visceral aspect of the pancreas there are multiple coalesced areas of pallor.	OP: 27.89 (M-gene), 24.25 (HP H5), 26.77 (N1) CP: 29.74 (M-gene), 26.06 (HP H5), 28.78 (N1)	
	5/7/2022		Bird 3	Noss	Female	Frozen	Moderate	Good	Crop, proventriculus and gizzard are empty. Scant digestive or fecal material in the intestine and colon.	OP: 29.71 (M-gene), 27.31 (HP H5), 29.54 (N1) CP: No Cq (M-gene), No Cq (HP H5), No Cq (N1)	
	5/1/2022		Bird 4	No Ness	Female	Frozen	Moderate	Good	Empty crop and no feed in the gizzard.	OP: 22.63 (M-gene), 20.91 (HP H5), 22.79 (N1) CP: 25.25 (M-gene), 22.29 (HP H5), 24.47 (N1)	
	5/1/2022		Bird 5	No Ness	Female	Frozen	Moderate	Good	Empty crop and no feed in the gizzard.	OP: 27.41 (M-gene), 24.39 (HP H5), 26.89 (N1) CP: 29.88 (M-gene), 25.99 (HP H5), 28.39 (N1)	
5/20/2022	5/9/2022	Great skua	Bird 1	Hermaness NNR	Female	Frozen	Moderate	Good	Multifocal concaved areas of dark discoloration at proventriculus-gizzard junction, no feed content seen.	Not performed	
	5/2/2022		Bird 2	Fair Isle	Female	Frozen	Moderate	Good	Empty gizzard		
	5/1/2022		Bird 3	Fair Isle	Male	Frozen	Moderate	Good	Empty gizzard		
8/5/2022	5/23/2022	Long tailed skua	n/a	Clumlie	Male	Frozen	Moderate	Good	Empty gizzard and no digesta within intestinal content.	Not performed	All positive (see tissue PCR tab)
6/20/2022	6/19/2022	Herring gull	Bird 1	Hastings, East Susex	ND	Fresh	Mild	Fair	Pancreas –multifocal to coalescent tan faint discoloration. In the gizzard, there are multiple nematodes of approximately 1 cm to 2 cm.	OP: 22.38 (M-gene), 21.66 (HP H5), 24.34 (N1) CP: 25.69 (M-gene), 25.71 (HP H5), 27.99 (N1)	Pooled brain: 15.27 (M-gene), 15.23 (HP H5), 17.24 (N1) Pooled intestines: 21.3 (M-gene), 18.97 (HP H5), 20.38 (N1) Pooled trachea and lung: 20.2 (M-gene), 18.14 (HP H5), 19.77 (N1) Pooled viscera: 22.6 (M-gene), 20.15 (HP H5), 21.16 (N1)
			Bird 2		ND	Fresh	Mild	Fair	Pancreas has multifocal tan faint discoloration. Spleen is mildly enlarged with the enhanced follicular pattern.	OP: 23.66 (M-gene), 22.48 (HP H5), 25.2 (N1) CP: 24.55 (M-gene), 24.56 (HP H5), 25.92 (N1)	
			Bird 3		ND	Fresh	Mild	Fair	Pancreas has multifocal to coalescent tan faint discoloration.	OP: 24.76 (M-gene), 24.54 (HP H5), 26.91 (N1) CP: 26.83 (M-gene), 26.98 (HP H5), 29.36 (N1)	Not tested

8/18/2022	8/17/2022	Herring gull	Bird 1	Penzance, Cornwall	Male	Fresh	Mild	Fair	Ingesta and digesta present in the digestive tract but the fecal material is runny. Pancreas has multifocal tan discoloration (necrosis). Mild splenic enlargment. Slight prominence of meningeal vessels.	OP: 28.46 (M-gene), 28.35 (HP H5), 30.22 (N1) CP: 32.74 (M-gene), 32.54 (HP H5), 34.44 (N1)	Pooled brain: 18.53 (M-gene), 18.84 (HP H5), 20.93 (N1) Pooled intestines: 22.92 (M-gene), 22.09 (HP H5), 24.08 (N1) Pooled trachea and lung: 19.88 (M-gene), 19.03 (HP H5), 20.61 (N1) Pooled viscera: 20.5 (M-gene), 19.57 (HP H5), 20.77 (N1)
			Bird 2		Male	Fresh	Mild	Fair	Ingesta and digesta present in the digestive tract but the fecal material is runny. Multifocally in the pancreas has tan discoloration (necrosis). Mild splenic and renal enlargement. Slight prominence of meningeal vessels.	OP: 29 (M-gene), 28.67 (HP H5), 29.84 (N1) CP: 34.5 (M-gene), 33.62 (HP H5), 37.27 (N1)	
3/8/2022	1/28/2022	Black-headed gull	Bird 1	Birmingham, West Midlands	Male	Frozen	Marked	Fair	Advanced autolysis	OP: 26.73 (M-gene), 24.48 (HP H5), 29.90 (N1) CP: 25.55 (M-gene), 23.65 (HP H5), 27.7 (N1)	Not performed
			Bird 2		Male	Frozen	Marked	Fair	Advanced autolysis	OP: 24.89 (M-gene), 23.46 (HP H5), 31.64 (N1) CP: 25.63 (M-gene), 24.22 (HP H5), 29.16 (N1)	
			Bird 3		Female	Frozen	Marked	Fair	Advanced autolysis	OP: 26.00 (M-gene), 23.57 (HP H5), 29.13 (N1) CP: 27.70 (M-gene), 25.24 (HP H5), 31.01 (N1)	
			Bird 4		Female	Frozen	Marked	Fair	Advanced autolysis	OP: 22.11 (M-gene), 21.18 (HP H5), 25.92 (N1) CP: 22.20 (M-gene), 19.56 (HP H5), 25.61 (N1)	

ND, not determined; PCR, polymerase chain reaction

Supplemental Table S2. Description of histologic scoring system

Organ	No significant lesion (-)	Grade			
		Minimal (+)	Mild (++)	Moderate (+++)	Severe (++++)
Skin*	No significant changes	Scattered lympho/plasmacytic infiltration within the dermis, unremarkable epidermis	Occasional vascular endothelial necrosis, multifocal clusters of lymphocytes/plasma cells within the dermis	Multiple vascular necrosis +/- fibrin deposition, multifocal clusters of lymphocytic/plasmacytic/histiocytic infiltration within the dermis +/- epidermal degeneration/necrosis	Epidermal degeneration & necrosis, dermal vascular necrosis, lympho/plasmacytic/histiocytic dermatitis
Skeletal muscle		Scattered lympho/plasmacytic infiltration between myofibers or interstitial spaces	Clusters of myofiber degeneration (myocyte swelling and loss of cross-striations) with lymphocytic/plasmacytic/histiocytic infiltration between myofibers or interstitial spaces	Multifocal myofiber necrosis (myocytes fragmentation, hypereosinophilic sarcoplasm, loss of cross striations and pyknotic / karyorrhectic nuclei), lymphocytic/plasmacytic/histiocytic infiltration	Myofiber necrosis, histiocytes phagocytosed cellular debris, replaced lost myofibers by cellular debris, fibrin deposition, lymphocytic/plasmacytic infiltration
Heart		Scattered lympho/plasmacytic infiltration between myofibers or interstitial spaces	Clusters of myofiberdegeneration (myocyte swelling and loss of cross striations) with lymphocytic/plasmacytic/histiocytic infiltration between myofibers or interstitial spaces	Multifocal myofiber necrosis (myocytes fragmentation, hypereosinophilic sarcoplasm, loss of cross striations and pyknotic / karyorrhectic nuclei), lymphocytic/plasmacytic/histiocytic infiltration	Myofiber necrosis, histiocytes phagocytosed cellular debris, replaced lost myofibers by cellular debris, fibrin deposition, lymphocytic/plasmacytic infiltration
Brain		Scattered necrosis (pyknosis /karyorrhexis) of neuronal or glial cells (1 to 3 cells)	Multiple clusters of necrotic neuronal or glial cells (each foci 4-9 cells), inflammatory infiltration and rarefaction of the neuropil, +/- meningitis	Multifocal areas of neuronal necrosis (each foci more than 10 affected cells), gliosis, perivascular lymphocytic/plasmacytic/histiocytic cuffing, +/- meningitis	Coalescing areas of neuronal necrosis, gliosis, thrombotic vessels, perivascular lymphocytic/plasmacytic/histiocytic cuffing, +/- meningitis
Spleen		Mild lymphoid depletion but retaining white pulp architecture	Multiple clusters of necrotic splenocytes with apparent lymphoid depletion of the white pulp	Multiple clusters of necrotic splenocytes with tingible body macrophages, fibrin deposition in parenchyma, partial loss of white pulp architecture	Complete lymphoid depletion with the replacement of white pulp with lytic/karyorrhectic debris, no discernable white pulp
Kidney		Scattered lympho/plasmacytic infiltration within the renal interstitium	Occasional degenerated cells (cytoplasmic eosinophilia, nuclear pknosis), clusters of lymphocytic/plasmacytic/histiocytic infiltration	Segmental necrotic tubular epithelium with karyorrhectic debris, heterophils, lymphocytic/plasmacytic/histiocytic infiltration	Confluent areas of necrotic tubular epithelium with karyorrhectic debris, heterophils, lymphocytic/plasmacytic/histiocytic infiltration, +/- fibrin deposition
Nasal turbinate **		Scattered lymphocytic /plasmacytic infiltration of the submucosa and/or mucosa	Multifocal loss of cilia but with intact mucosal epithelium, heterophilic exocytosis, multifocal clusters of lymphocytic/plasmacytic/histiocytic infiltration of the mucosa and/or submucosa	Multifocal necrosis of the mucosal epithelium, clusters of intraluminal exudate, diffuse lymphocytic/ plasmacytic/ histiocytic infiltration of the mucosa and/or submucosa	Complete loss of mucosal epithelium +/- fibrin deposition or hemorrhage, submucosal lymphocytic/plasmacytic/histiocytic infiltration, fibrinoid necrosis/thrombosis of the blood vessels
Trachea **		Scattered lymphocytic/plasmacytic infiltration of the submucosa and/or mucosa	Multifocal loss of cilia but with intact mucosal epithelium, heterophilic exocytosis, multifocal clusters of lymphocytic/plasmacytic/histiocytic infiltration of the mucosa and/or submucosa	Multifocal necrosis of the mucosal epithelium, clusters of intraluminal exudate, diffuse lymphocytic/plasmacytic/histiocytic infiltration of the mucosa and/or submucosa	Complete loss of mucosal epithelium +/- fibrin deposition, submucosal lymphocytic/plasmacytic/histiocytic infiltration, fibrinoid necrosis/thrombosis of the blood vessels
Lung		Scattered lymphocytic/plasmacytic infiltration within the air capillaries or peribronchial spaces	Multifocal expansion of the air capillaries or peribronchial spaces by lymphocytic/ plasmacytic/ histiocytic infiltration	Multiple clusters of necrotic cells within the air capillaries or peribronchial spaces, expansion of the air capillaries or peribronchial spaces by lymphocytic/plasmacytic/histiocytic infiltration	Confluent necrotizing broncho/interstitial pneumonia +/- fibrin deposition, lymphocytic/plasmacytic/histiocytic infiltration of the interstitial spaces
Proventriculus		Scattered lymphocytic/plasmacytic infiltration within the submucosa/lamina propria, or ganglioneuritis	Mild expansion of the lamina propria +/- submucosa by lymphocytic/plasmacytic/histiocytic infiltration, +/- ganglioneuritis, unremarkable epithelium	Multifocal necrosis of the mucosa epithelium and/or glandular epithelium, lymphocytic/plasmacytic/histiocytic infiltration of the lamina propria/submucosa, +/- ganglioneuritis	Confluent loss of epithelium +/- fibrin deposition or hemorrhage, submucosal lymphocytic/plasmacytic/histiocytic infiltration, fibrinoid necrosis/thrombosis of the blood vessels, +/- ganglioneuritis
Gizzard		Scattered lymphocytic/plasmacytic infiltration within the submucosa/lamina propria, or ganglioneuritis	Mild expansion of the lamina propria +/- submucosa by lymphocytic/plasmacytic/histiocytic infiltration, +/- ganglioneuritis, unremarkable epithelium	Multifocal necrosis of the mucosa epithelium with koilin fragmentation, lymphocytic/plasmacytic/histiocytic infiltration of the lamina propria/submucosa, +/- ganglioneuritis	Confluent loss of epithelium with detachement of koilin, fibrin deposition and/or hemorrhage, submucosa lymphocytic/plasmacytic/histiocytic infiltration, fibrinoid necrosis/thrombosis of the blood vessels, +/- ganglioneuritis
Liver		Single cell necrosis involving less than 10% of the examined hepatic tissue, +/- lympho/plasmacytic infiltration	Multifocal areas of hepatic necrosis involving between 10 to less than 25% of the examined hepatic tissue, +/- lymphocytic/plasmacytic/histiocytic infiltration	Multifocal areas hepatic necrosis involving between 25 to less than 50% of the examined hepatic tissue, +/- lymphocytic/plasmacytic/histiocytic infiltration	Extensive, coalesced areas of hepatic necrosis involving more than 50% of the examined hepatic tissue, +/- lymphocytic/plasma/histiocytic infiltration
Pancreas		Occasional foci of pancreatic necrosis involving less than 10% of the examined pancreatic tissue, +/- lymphocytic/plasmacytic infiltration	Multifocal areas of pancreatic necrosis involving between 10 to less than 25% of the examined pancreatic tissue, +/- lymphocytic/plasmacytic/histiocytic infiltration	Multifocal areas pancreatic necrosis involving between 25 to under 50% of the examined pancreatic tissue, +/- lymphocytic/plasmacytic/histiocytic infiltration	Extensive, coalesced areas of pancreatic necrosis involving more than 50% of the examined pancreatic tissue, +/- lympho/plasma/histiocytic infiltration

Duodenum		Scattered lymphoplasmacytic cellular infiltrate within the submucosa/lamina propria, or ganglioneuritis	Mild expansion of the lamina propria +/- submucosa by lymphoplasmacytic cell infiltration, +/- ganglioneuritis, unremarkable epithelium	Multifocal necrosis of the epithelium, moderate expansion of the lamina propria+/- submucosa by lymphoplasmacytic cell infiltration, +/- ganglioneuritis	Complete loss of epithelium +/- fibrin deposition or hemorrhage, submucosa lymphoplasmacytic infiltration, fibrinoid necrosis/thrombosis of the blood vessels, +/- ganglioneuritis
Ovary		Occasional foci of necrosis involving less than 10% of the examined ovarian tissue (including follicles, interstitial connective tissues, blood vessels), +/- lymphocytic/plasmacytic infiltration	Multifocal areas of necrosis involving between 10 to less than 25% of the examined ovarian tissue, +/- lymphocytic/plasmacytic/histiocytic infiltration	Multifocal areas necrosis involving between 25 to less than 50% of the examined ovarian tissue, +/- lymphocytic/plasmacytic/histiocytic infiltration	Extensive, coalesced areas of necrosis involving more than 50% of the examined ovarian tissue, +/- lymphocytic/plasma/histiocytic infiltration
Oviduct		Scattered lymphocytic/plasmacytic cellular infiltrate within the submucosa	Mild expansion of the submucosa by lymphoplasmacytic cell infiltration, unremarkable epithelium	Multifocal necrosis of the epithelium, moderate expansion of the submucosa by lymphoplasmacytic cell infiltration	Complete loss of epithelium +/- fibrin deposition or haemorrhage, submucosa lymphoplasmacytic infiltration, fibrinoid necrosis/thrombosis of the blood vessels, +/- ganglioneuritis
Testis		Occasional foci of involving less than 10% of the examined testicular tissue (including seminiferous tubules, interstitial connective tissues, blood vessels), +/- lymphocytic/plasmacytic infiltration	Multifocal areas of necrosis involving between 10 to less than 25% of the examined testicular tissue, +/- lymphocytic/plasmacytic /histiocytic infiltration	Multifocal areas necrosis involving between 25 to under 50% of the examined testicular tissue, +/- lymphocytic/plasmacytic/histiocytic infiltration	Extensive, coalesced areas of necrosis involving more than 50% of the examined testicular tissue, +/- lymphocytic/plasmacytic/histiocytic infiltration

* feather pulp or follicle not examined
** underlying bone or cartilage not assessed

Supplemental Table S3. Description of the immunohistochemistry (IHC) scoring matrix

IHC Grade	Terminology	Definition
-	Absent	No labeling
+	Rare	Sparse labeling, single isolated cells
++	Scattered	Clusters of 2-5 positive cells
+++	Confluent	Clusters of >6 cells, still well distinct foci of labeling
++++	Abundant	Diffuse, coalesced areas of labeling

Supplemental Table S4. Case descriptions of disease outbreaks in herring gulls.

Case Ref	Description
Herring gull 19/06/2022	Suspicion of notifiable avian disease has been reported in a group of 103 herring gulls at a wildlife rehabilitation center. Clinical signs first noted in the group on the 18/06/2022, this included respiratory signs, gasping and muscle twitching. One bird died on Saturday, 15 Sunday (8 died and 7 euthanised) and 6 today (2 died and 4 euthanised). Within the affected building there is a separate area currently housing 61 captive birds of 17 different species. All other species are reported to be unaffected. There is also a separate unaffected building onsite housing 10 mammals and approximately 40 captive birds of mixed species. An APHA vet carried out a thorough investigation and confirmed the mortality numbers. The mortality is confined in the herring gulls' younger group(nestlings) only and two more birds were found dead at the time of the investigation, bringing the total number of deaths up to 8 birds today. The affected birds have neurological and respiratory signs. There are also approx. 100 birds of other species on site. Older herring gulls as well as the birds of other species are reported to be clinically normal and there are no deaths in these groups. Mortality is 24/103 herring gulls.
Herring gull 17/08/2022	Keepers reported suspicion of Avian Notifiable Disease in wild bird hospital. 15 herring gulls died yesterday and a further 20 have died today, showing respiratory and nervous signs along with diarrhoea prior to death. The hospital has gulls, pigeons and garden birds on site in indoor and outdoor pens and is located on the coast. The hospital has 160 wild birds including gulls, pigeon, garden bird An APHA vet investigated and confirmed increased mortality in the gulls' group, all kept in one pen, with 15 dead on the 17th August and approx. 32 dead birds on the 18th. The gulls are off their feed, at least 6 have cyanotic heads and some birds have diarrhoea. The rest of the birds at the premises are kept in separate pens and all appear to be clinically normal.

Supplemental Table S5. Polymerase chain reaction Cq values in various tissues of great skua. * Cq ≤36.0 were considered positive

Great Skua (16/05/2022)															
	Bird 1			Bird 2			Bird 3			Bird 4			Bird 5		
	M-gene	H5 HP-gene	N1-gene	M-gene	H5 HP-gene	N1-gene	M-gene	H5 HP-gene	N1-gene	M-gene	H5 HP-gene	N1-gene	M-gene	H5 HP-gene	N1-gene
Brain	23.78	18.58	20.56	25.57	21.95	24.74	22.09	19.20	24.74	18.63	14.97	16.84	20.92	17.08	19.59
Trachea	26.82	24.46	26.17	28.59	25.98	28.82	No Cq	33.41	35.01	26.70	23.09	25.45	26.71	23.26	25.12
Lung	22.47	20.15	22.12	23.75	20.15	22.22	28.19	25.15	27.56	26.40	20.67	21.88	36.28	21.64	20.51
Heart	26.07	21.35	23.97	24.96	22.12	24.83	30.45	27.55	29.71	21.25	17.33	18.94	20.85	16.79	19.48
Liver	26.27	20.81	23.22	30.76	22.62	24.91	30.17	26.54	28.56	25.28	19.79	22.05	23.94	18.36	20.50
Spleen	26.28	22.56	24.84	26.05	24.20	27.21	27.36	24.60	26.71	No Cq	No Cq	No Cq	24.31	19.45	20.99
Kidney	27.13	24.06	25.57	24.40	19.81	22.13	27.61	24.49	26.23	23.02	17.91	20.04	23.44	17.87	19.85
Intestine	25.61	22.76	25.00	24.23	20.64	23.81	29.49	26.46	28.55	24.79	21.49	23.04	26.16	20.82	22.65

Great Skua (20/05/2022)									
	Skua 1			Skua 2			Skua 3		
	M-gene	H5 HP-gene	N1-gene	M-gene	H5 HP-gene	N1-gene	M-gene	H5 HP-gene	N1-gene
Brain	19.97	16.83	20.41	25.73	22.16	25.78	19.39	17.51	21.49
Trachea	21.42	19.01	22.70	27.96	25.33	28.00	34.79	31.04	33.83
Lung	22.56	18.03	20.49	25.90	22.41	24.90	26.41	23.75	26.03
Heart	20.50	16.86	20.27	28.77	25.19	27.56	28.48	25.83	28.89
Liver	24.71	20.32	23.73	32.12	29.12	31.39	28.59	25.61	29.29
Spleen	24.43	20.81	23.41	30.46	26.39	28.08	28.11	25.30	28.27
Kidney	22.14	17.62	21.37	27.69	22.60	25.24	28.62	24.49	27.95
Intestine	26.82	24.25	26.73	29.23	27.08	29.42	26.64	24.17	27.46

	Long-tailed skua (23/05/2022)		
	M-gene	H5 HP-gene	N1-gene
Brain	23.00	20.74	24.69
Trachea	Not sampled		
Lung	21.70	18.50	23.25
Heart	21.61	17.88	20.79
Liver	21.02	17.48	22.27
Spleen	Not sampled		
Kidney	22.70	17.12	22.49
Intestine	25.44	22.69	27.26

Supplemental Table S6. Influenza virus tissue tropism characterized by immunohistochemistry

Bird Species	Skua (16/05/22)					Skua (20/05/22)			Long tailed skua (05/08/22)	Herring gull (20/06/22)			Herring gull (18/08/22)		Black headed gull (08/03/22)			
Bird ID	**Bird 1**	**Bird 2**	**Bird 3**	**Bird 4**	**Bird 5**	**Bird 1**	**Bird 2**	**Bird 3**	**Bird 1**	**Bird 1**	**Bird 2**	**Bird 3**	**Bird 1**	**Bird 2**	**Bird 1**	**Bird 2**	**Bird 3**	**Bird 4**
Skin	-	V	V	V	V	V	-	-	V	-	-	-	-	n/a	-	-	-	V
Skeletal Muscle	V>M	M>V	M	M,V	V	V	M	-	V	M	M	M	M	M	M	n/a	M	V>M
Heart	V>M	M	M	V>M	V>M	M>V	M	M	M, V	M, V	M	M	M	M,V	M	M	M, V	V>M
Brain	N, V	V>N	N	N, V	N, V	N, V	N>V	N>V	V>N	N>V	N>Epd	N>Epd	N	N>V	N>V	N>V	N>V	V>N
Spleen	V, R	n/a	V, R	n/a	V, R	n/a	-	n/a	V, R	R>V	R	R>V	R	R	n/a	n/a	n/a	n/a
Kidney	Ep,V	Ep>V	Ep	Ep>V	V>Ep	Ep, V	Ep	Ep	Ep, V	V	-	Ep	-	Ep, V	n/a	n/a	n/a	Ep, V
Turbinates	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	R>V	Ep,V,R	Ep>V	Ep	n/a	n/a	n/a	n/a	n/a
Trachea	V	V	-	V	V	V	-	-	V	Ep	Ep	Ep	n/a	n/a	n/a	n/a	n/a	n/a
Lung	Ep, V	Ep, V	Ep, V	Ep, V	Ep, V	Ep, V	Ep, V	Ep, V	Ep, V	Ep, V	Ep, V	Ep, V	Ep	Ep, V	n/a	n/a	Ep, V	Ep, V
Proventriculus	V	V	-	V>Ep	V	V	Ep	-	n/a	V	-	-	-	-	-	-	V	V>E
Gizzard	V, Ep	V	-	V	V	V	-	-	V	-	-	Ep, R	-	-	V, R	n/a	V	V
Liver	V, R, Ep	R, V	Ep	V, R, E	V	V	-	Ep	V	Ep, V	-	Ep>V	R	R	-	n/a	-	R, V
Pancreas	Ep, V	Ep, V	Ep	Ep, V	Ep, V	Ep>V	Ep	Ep	Ep, V	Ep>V	Ep>V	Ep>V	Ep>V	Ep>V	n/a	n/a	n/a	n/a
Duodenum	V, R	V	-	V	V	V>Ep	-	-	n/a	V, R	-	V, R	-	V, R	n/a	n/a	n/a	n/a
Ovary	E, V, ME	E, V, ME	Ep	Ep, V, ME	Ep, V, ME	Ep, V, ME	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	ME, V
Oviduct	Ep>V	Ep	Ep	E>V,R	E>V	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Testis	n/a	n/a	n/a	n/a	n/a	n/a	-	-	V>Ep	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Ep, epithelial cells; V, vascular endothelial cells; N, neuronal cells; Epd, ependymal cells; R, round cells (macrophages, dendritic cells); M, myocytes (cardiac/ skeletal/ smooth); ME, mesenchymal cells (fibroblasts, adipocytes); n/a, not available; >, more abundant than;