

# Highly Pathogenic Avian Influenza Virus A(H5N1) Clade 2.3.4.4b Infection in Free-Ranging Polar Bear, Alaska, USA

Raphaela Stimmelmayer,<sup>1</sup> David Rotstein,<sup>1</sup> Mia Kim Torchetti, Robert Gerlach

We report a natural infection with a Eurasian highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus in a free-ranging juvenile polar bear (*Ursus maritimus*) found dead in North Slope Borough, Alaska, USA. Continued community and hunter-based participation in wildlife health surveillance is key to detecting emerging pathogens in the Arctic.

Since its emergence in Europe during October 2020, highly pathogenic avian influenza (HPAI) A(H5N1) clade 2.3.4.4b virus has frequently spilled over into diverse mammal hosts globally. In North America, natural H5N1 infections have occurred in several bear species, including American black bears (*Ursus americanus*), Asiatic black bears (*U. thibetanus*), grizzly bears (*U. arctos horribilis*), and Kodiak brown bears (*U. a. middendorffi*) (1). Infections with influenza A(H1N1) viruses have been reported in captive sloth bears (*Melursus ursinus*) and Asiatic black bears (2,3) and in giant pandas (*Ailuropoda melanoleuca*) (4). Detection of hemagglutination inhibition antibodies against H3 and H6 subtype influenza viruses also suggested previous natural exposure to influenza viruses of avian origin (4). Seroconversion after natural exposure to bird influenza viruses has been documented in the Barent Sea polar bear subpopulation (2010–2011) (5) and brown bears in Alaska (2013–2016) (6) but not in the southern Beaufort Sea polar

bear subpopulation (2013–2016) (7). Polar bears are a threatened species under the US Endangered Species Act. We report and describe an infection by HPAI H5N1 virus in a free-ranging polar bear found dead in Alaska, USA, during 2023.

## The Study

The North Slope Borough Department of Wildlife Management (NSB DWM) in Alaska conducts wildlife health research and maintains community-based harvest monitoring programs for marine mammals, including polar bears. The Alaska Office of the State Veterinarian conducts surveillance for notifiable infectious diseases in wildlife. After detecting HPAI H5N1 in birds of prey and a red fox (*Vulpes vulpes*) in April 2022, the Office of the State Veterinarian initiated collaborative surveillance testing with NSB DWM for avian influenza in birds and other wildlife. In August 2023, community members reported a dead polar bear without obvious external injuries near Point Barrow, Alaska (71°23'N, 156°28'W). At the NSB DWM laboratory in Utqiagvik, Alaska, we conducted a post-mortem examination of the bear. The bear was young and male, 120 cm in body length, and in moderate to advanced decomposition. Body condition was fair to poor, with no back or visceral fat. Gross findings were multiple 1–3-cm ulcerative skin lesions around the left eye and oral commissure, liver and lung congestion, moderate sanguinal pericardial and cavitory effusion, cerebral swelling and congestion, and empty stomach. We collected postmortem tissue samples of the heart, lung, trachea, spleen, liver, kidney, adrenal gland, skin, skeletal muscle, mesenteric lymph node, pancreas, tongue, esophagus, stomach, small intestines, and brain (cerebrum) and fixed them in 10% neutral buffered formalin for 2 weeks. Histology

Author affiliations: North Slope Borough, Utqiagvik, Alaska, USA (R. Stimmelmayer); University of Alaska, Fairbanks, Alaska, USA (R. Stimmelmayer); Marine Mammal Pathology Services, Olney, Maryland, USA (D. Rotstein); US Department of Agriculture Animal and Plant Health Inspection Service, Ames, Iowa, USA (M.K. Torchetti); Alaska Department of Environmental Conservation, Anchorage, Alaska, USA (R. Gerlach)

DOI: <https://doi.org/10.3201/eid3008.240481>

<sup>1</sup>These authors contributed equally to this article.

Consultation Services (<https://histocs.com>) processed the tissue for routine histopathologic examination by staining with hematoxylin and eosin. We also collected oral, nasal, rectal, and brain swab samples and placed them in 2-mL cryovials, which we stored for 2 weeks at  $-50^{\circ}\text{C}$  and shipped to the Alaska Environmental Health Laboratory in Anchorage, Alaska. Their personnel placed pooled swab specimens into brain-heart infusion broth. The primary histopathologic finding was a granulocytic and mononuclear meningoencephalitis with microgliosis, neuronal necrosis, neuronophagia, vasculitis, and parenchymal rarefaction (Figure, panel A). Other findings were pulmonary edema, focal lipid pneumonia, and multifocal ulcerative dermatitis.

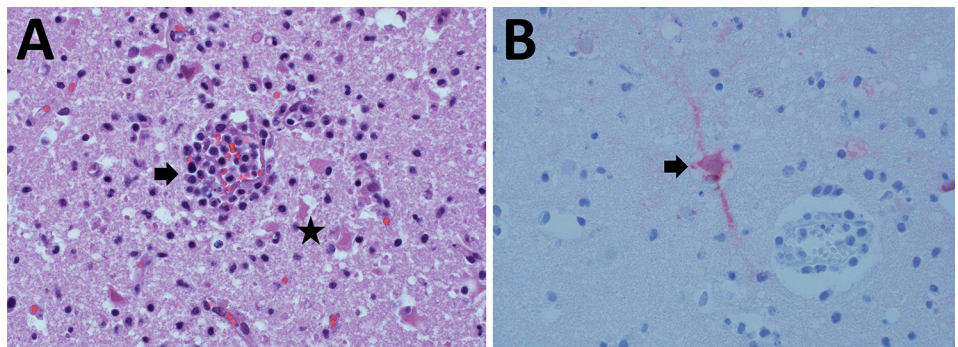
Pooled swab specimens tested negative for the influenza virus matrix gene by PCR at the Washington Animal Disease Diagnostic Laboratory (Pullmans, WA, USA), which is a National Animal Health Laboratory Network facility. The root cause of negative PCR results is unclear because subsequent sequence analysis did not indicate assay failure. However, because of the cerebral lesions, we sent scrolls of formalin-fixed paraffin-embedded cerebral tissue to the Athens Disease Diagnostic Laboratory, University of Georgia (Athens, GA, USA), for immunohistochemistry to detect influenza A by using an influenza A virus polyclonal antibody (Abcam, <https://www.abcam.com>). Influenza A virus antigen was detected in cytoplasm of neurons and nuclei of microglial cells (Figure, panel B). We also sent scrolls of formalin-fixed paraffin-embedded cerebral tissue to the National Veterinary Services Laboratories (Ames, IA, USA), for molecular confirmation and virus genome characterization. HPAI virus genotype A3, a fully Eurasian influenza virus, was identified; this genotype was initially detected in Alaska in April 2022 and was the most frequently detected genotype in Alaska during August–December 2023. Reported markers for mammal

adaptation were not identified. We deposited full genome sequences for the polar bear virus (A/polar bear/Alaska/23-0381234/2023) in GenBank (accession nos. PP820319–26) and GISAID (<https://www.gisaid.org>; accession no. EPI\_ISL\_18976667).

This detection of HPAI virus was in the southern Beaufort Sea subpopulation, 1 of 19 circumpolar polar bear subpopulations. During July–August 2023, three short-tailed shearwater seabirds (*Ardenna tenuirostris*) that tested positive for HPAI H5N1 clade 2.3.4.4b were found dead near Point Barrow, where the polar bear in this study was found. The shearwaters' virus genotype shared 9 common single-nucleotide polymorphisms (SNPs) with the polar bear virus and was representative of the virus circulating in that area at the time rather than a direct source of the polar bear infection. In addition, in August 2023, a small mortality event from avian influenza occurred among common murre seabirds (*Uria aalge*) in Dillingham Census Area in Alaska; that virus genotype also shared 8–9 common SNPs, further supporting regional virus circulation. Polar bears are primarily dependent on seals as a food source but will prey on birds and eggs; thus, virus exposure from consumption of infected birds is possible, but infection via an olfactory route cannot be excluded (8).

Support does not exist for ongoing HPAI virus-associated illness and death in free-ranging polar bears in Alaska's North Slope Borough; in 2023, swab specimens from 3 other dead polar bears tested negative for influenza virus by PCR (Appendix, <https://wwwnc.cdc.gov/EID/article/30/8/24-0481-App1.pdf>). As for black bears with HPAI H5N1 virus infections (9), brain lesions were the major histopathologic findings in this case. It is not unexpected for clade 2.3.4.4b virus-infected mammals with neurologic signs to have respiratory samples test negative (10), possibly because of different exposure routes, such as digestive, olfactory, or respiratory routes (8). The HPAI H5 goose/Guangdong lineage has been shown to be more neuropatho-

**Figure.** Histologic analysis of brain tissue from a dead free-ranging polar bear infected with highly pathogenic avian influenza virus A(H5N1) clade 2.3.4.4b, Alaska, USA. A) Hematoxylin and eosin staining of brain tissue section showing meningoencephalitis. Arrow indicates mixed inflammatory cells within and around blood vessels and hypertrophied vascular endothelial cells. Star indicates necrotic neurons and increased number of microglial cells within the parenchyma. Original magnification  $\times 400$ . B) Arrow indicates influenza A virus within the neuronal perikaryon (red staining) observed by immunohistochemistry of formalin-fixed paraffin-embedded brain sections. Original magnification  $\times 400$ .



genic than other influenza A viruses in mammals (8), including the H5N1 clade 2.3.4.4b virus (11,12).

Other notable findings in this case were pulmonary edema, lipid pneumonia, and ulcerative skin lesions. Pulmonary edema as a gross lesion and on histologic analysis was a consistent finding in 3 domestic cats with H5N1 clade 2.3.4.4b infections (13) but has been infrequently reported in wild mesocarnivores (10). Lipid pneumonia was documented in subsistence-harvested polar bears in the southern Beaufort Sea and is considered unrelated (D. Rotstein, unpub. data). Ulcerative skin lesions not caused by trauma are rarely documented in subsistence-harvested southern Beaufort Sea polar bears (R. Stimmelmayer, unpub. data); those lesions have not been reported in terrestrial mammals infected with the H5 clade 2.3.4.4b lineage (10) but have been reported in pinnipeds infected with H3N8 virus (14).

## Conclusions

Genome analysis of influenza viruses originating from wildlife in Alaska has shown both un reassorted and reassorted viruses. HPAI virus genotype A3 was likely introduced into Alaska via the East Asia–Australia Flyway as early as November 2021 (15) and has been detected in a few backyard premises; in many wild birds, including California condors (*Gymnogyps californianus*) in Arizona; and several mammals (red fox, fishers, martens, racoons, and brown bears) along the Pacific Flyway.

In the Arctic, wildlife and other wild subsistence foods play a pivotal role in the health, well-being, and food security of northern indigenous communities. Therefore, subsistence harvesting of animals infected with HPAI viruses, including polar bears, poses a zoonotic risk and affects traditional food safety and food security. Continued community and hunter-based participation in wildlife health surveillance is key to detecting emerging pathogens and other One Health issues in the Arctic.

## Acknowledgments

We thank the hunters and communities in the North Slope Borough who keep us informed about what they see when traveling the ocean and land; all of our subsistence and wildlife research assistant staff, as well as department leadership for their continued support of the NSB DWM wildlife health research program; Hon Ip for his support during the investigation; Histology Pathology Services for their slide preparation and rapid responsiveness; and staff in the Histology Laboratory in the Department of Pathology at the University of Georgia College of Veterinary Medicine for their immunohistochemistry expertise.

Marine mammal tissues were collected under US Fish and Wildlife Service (permit no. MA80164B-0).

The findings and conclusions in this publication are those of the authors and should not be construed to represent any official US Department of Agriculture or US Government determination or policy.

## About the Author

Dr. Stimmelmayer is a wildlife veterinarian and research biologist with the North Slope Borough Department of Wildlife Management in Utqiagvik, Alaska. Her research interests focus on marine and terrestrial wildlife health and diseases within a One Health context of indigenous circumpolar hunting societies.

## References

1. US Department of Agriculture, Animal and Plant Health Inspection Service. Detections of highly pathogenic avian influenza in mammals [cited 2024 Feb 12]. <https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/mammals>
2. Boedeker NC, Nelson MI, Killian ML, Torchetti MK, Barthel T, Murray S. Pandemic (H1N1) 2009 influenza A virus infection associated with respiratory signs in sloth bears (*Melursus ursinus*). *Zoonoses Public Health*. 2017;64:566–71. <https://doi.org/10.1111/zph.12370>
3. Bessière P, Gaide N, Croville G, Crispo M, Fusade-Boyer M, Abou Monsef Y, et al. High pathogenicity avian influenza A(H5N1) clade 2.3.4.4b virus infection in a captive Tibetan black bear (*Ursus thibetanus*): investigations based on paraffin-embedded tissues, France, 2022. *Microbiol Spectr*. 2024;12:e0373623. <https://doi.org/10.1128/spectrum.03736-23>
4. Li D, Zhu L, Cui H, Ling S, Fan S, Yu Z, et al. Influenza A(H1N1)pdm09 virus infection in giant pandas, China. *Emerg Infect Dis*. 2014;20:480–3. <https://doi.org/10.3201/eid2003.131531>
5. Naidenko SV, Ivanov EA, Mordvintsev IN, Platonov NG, Ershov RV, Rozhnov VV. Seropositivity for different pathogens in polar bears (*Ursus maritimus*) from Barents Sea Islands. *Biol Bull Russ Acad Sci*. 2013;40:779–82. <https://doi.org/10.1134/S1062359013090082>
6. Ramey AM, Cleveland CA, Hilderbrand GV, Joly K, Gustine DD, Mangipane B, et al. Exposure of Alaska brown bears (*Ursus arctos*) to bacterial, viral, and parasitic agents varies spatiotemporally and may be influenced by age. *J Wildl Dis*. 2019;55:576–88. <https://doi.org/10.7589/2018-07-173>
7. Hemert CV, Spivey TJ, Uher-Koch BD, Atwood TC, Sinnott DR, Meixell BW, et al. Survey of Arctic Alaskan wildlife for influenza A antibodies: limited evidence for exposure of mammals. *J Wildl Dis*. 2019;55:387–98. <https://doi.org/10.7589/2018-05-128>
8. Bauer L, Benavides FFW, Veldhuis Kroeze EJB, de Wit E, van Riel D. The neuropathogenesis of highly pathogenic avian influenza H5Nx viruses in mammalian species including humans. *Trends Neurosci*. 2023;46:953–70. <https://doi.org/10.1016/j.tins.2023.08.002>
9. Jakobek BT, Berhane Y, Nadeau MS, Embury-Hyatt C, Lung O, Xu W, et al. Influenza A(H5N1) virus infections in 2

- free-ranging black bears (*Ursus americanus*), Quebec, Canada. *Emerg Infect Dis.* 2023;29:2145–9. <https://doi.org/10.3201/eid2910.230548>
10. Elsmo EJ, Wünschmann A, Beckmen KB, Broughton-Neiswanger LE, Buckles EL, Ellis J, et al. Highly pathogenic avian influenza A(H5N1) virus clade 2.3.4.4b infections in wild terrestrial mammals, United States, 2022. *Emerg Infect Dis.* 2023;29:2451–60. <https://doi.org/10.3201/eid2912.230464>
  11. Alkie TN, Cox S, Embury-Hyatt C, Stevens B, Pople N, Pybus MJ, et al. Characterization of neurotropic HPAI H5N1 viruses with novel genome constellations and mammalian adaptive mutations in free-living mesocarnivores in Canada. *Emerg Microbes Infect.* 2023;12:2186608. <https://doi.org/10.1080/22221751.2023.2186608>
  12. Bordes L, Vreman S, Heutink R, Roose M, Venema S, Pritz-Verschuren SBE, et al. Highly pathogenic avian influenza H5N1 virus infections in wild red foxes (*Vulpes vulpes*) show neurotropism and adaptive virus mutations. *Microbiol Spectr.* 2023;11:e0286722. <https://doi.org/10.1128/spectrum.02867-22>
  13. Sillman SJ, Drozd M, Loy D, Harris SP. Naturally occurring highly pathogenic avian influenza virus H5N1 clade 2.3.4.4b infection in three domestic cats in North America during 2023. *J Comp Pathol.* 2023;205:17–23. <https://doi.org/10.1016/j.jcpa.2023.07.001>
  14. Anthony SJ, St Leger JA, Puglianes K, Ip HS, Chan JM, Carpenter ZW, et al. Emergence of fatal avian influenza in New England harbor seals. *mBio.* 2012;3:e00166–12. <https://doi.org/10.1128/mBio.00166-12>
  15. Youk S, Torchetti MK, Lantz K, Lenoche JB, Killian ML, Leyson C, et al. H5N1 highly pathogenic avian influenza clade 2.3.4.4b in wild and domestic birds: introductions into the United States and reassortments, December 2021–April 2022. *Virology.* 2023;587:109860. <https://doi.org/10.1016/j.virol.2023.109860>

Address for correspondence: Raphaela Stimmelmayer, North Slope Borough, Department of Wildlife Management, PO Box 69, Ahkovak St 1795, Utqiagvik, AK 99723, USA; email: raphaela.stimmelmayer@north-slope.org

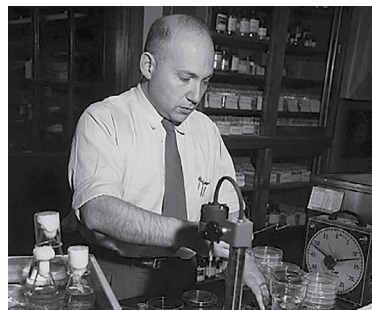
## etymologia

### Microbiota [mī''-krō-bī'-ō-'tə], microbiome [mī''-krō-bī'-ōm]

Julia Memrava Cabrera, Daniel F.M. Monte

From the Greek micro- (small) and -bios (life), microbiota was coined in the late 19th Century to denote the microorganisms residing in a specific environment. During the 20th Century, microbiota became more specifically associated with the microorganisms inhabiting the human body. Today, the term encompasses the collective genetic material of microorganisms, spanning viruses, archaea, bacteria, and fungi, and the intricate ecosystems of microorganisms, including commensal, symbiotic, and pathogenic ones, that exist within or on the human body or other environmental niches. Exploring microbiota and its implications in various aspects has rapidly gained momentum as a dynamic field of research.

The term microbiome was defined by Whipps and colleagues in 1988 as the collective genomes of microorganisms. However, Joshua Lederberg (Figure), a US molecular biologist, played a pivotal role in coining the term as we know it today. His journey from a precocious young scientist to a Nobel laureate and advocate for ethical science reflects the interconnectedness of language, curiosity, and scientific discovery. Lederberg's fascination with science



**Figure.** Molecular biologist and Nobel laureate Joshua Lederberg in his laboratory in Wisconsin, 1958. Dr. Lederberg played a pivotal role in coining the term microbiome as we know it today. Public domain image from the National Library of Medicine.

also extended to writing science fiction, using the genre to explore complex scientific concepts through imaginative storytelling. In fact, microbiome is a combination of microbe and biome (bi- [life] + -ome [mass]) to describe the microbial ecosystem, which encompasses not only genomes but also the broader microbial environment. Microbiome, born from the fusion of linguistic roots and a thirst for knowledge, continues to shape our understanding of the microbial world and its profound impact on human health and biology.

#### Sources

1. Dorland's illustrated medical dictionary. 32nd ed. Philadelphia: Elsevier Saunders; 2012.
2. Berg G, Rybakova D, Fischer D, Cernava T, Vergès MC, Charles T, et al. Microbiome definition re-visited: old concepts and new challenges. *Microbiome.* 2020;8:103. <https://doi.org/10.1186/s40168-020-00875-0>
3. Lederberg J, McCray AT. 'Ome sweet 'omics—a genealogical treasury of words. *Scientist.* 2001;15:8–9.
4. Liu X. Microbiome. *Yale J Biol Med.* 2016;89:275–6.
5. Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal. *Microbiome.* 2015;3:31. <https://doi.org/10.1186/s40168-015-0094-5>

Address for correspondence: Daniel F.M. Monte, Department of Animal Science, College for Agricultural Sciences, Federal University of Paraíba, Areia, 58397000, Brazil; email: monte\_dfm@alumni.usp.br

DOI: <https://doi.org/10.3201/eid3008.230677>

# Highly Pathogenic Avian Influenza Virus A(H5N1) Clade 2.3.4.4b Infection in Free-Ranging Polar Bear, Alaska, USA

## Appendix

### Additional Methods

#### 2023 Polar Bear Necropsies, North Slope Borough Department of Wildlife Management

A summary of avian influenza surveillance findings from 3 other polar bears found dead and 1 defense-of-life kill near Utqiagvik, Alaska, USA, collected during August–December 2023 is provided. Three of the 4 bears (polar bears no. 1–3) were found dead in the surf near Utqiagvik and 1 bear (polar bear no. 4) was a defense-of-life kill in Utqiagvik. No clinical signs of illness in polar bear number 4 were observed. Indigenous hunters salvaged the heads from polar bears 2 and 3. Postmortem examination of polar bear 1 (only external) and polar bear 4 (complete) was conducted by the wildlife veterinarian from the North Slope Borough Department of Wildlife Management. Tissue samples for routine histopathologic examination were fixed in 10% neutral-buffered formalin and processed for hematoxylin and eosin staining at the Histology Consultation Services (<https://histocs.com>) in Everson, Washington, USA. Swab specimens (i.e., oral, nasal, rectal, brain) were collected by using polyester swabs on plastic stems, placed in 2 mL cryovials, and stored frozen at  $-50^{\circ}\text{C}$ . At the Alaska Environmental Health Laboratory, swab specimens were pooled and placed in brain heart infusion broth before sending out for analysis.

#### PCR

Swab specimens were tested by PCR of the influenza A virus matrix gene at the Washington Animal Disease Diagnostic Laboratory, a member of the National Animal Health Laboratory Network (NAHLN). Members of NAHLN use the same PCR protocol as the National Veterinary Services Laboratories in Ames, Iowa. The subsequent sequence analysis for the highly pathogenic avian influenza virus–positive polar bear in this study did not point to any

issues with the PCR itself; therefore, we cannot resolve the root cause for the initial negative PCR test results for swab specimens. However, it is reasonable to conclude that the negative PCR results from swab specimens from the additional 3 polar bears found dead (see below) reflect true negatives and support ruling out suspicion of HPAI infection in those 3 bears.

## **IHC**

Influenza A virus immunohistochemistry was performed by staining formalin-fixed paraffin-embedded tissue sections with influenza A virus polyclonal antibody (Abcam, <https://www.abcam.com>). Immunohistochemistry was performed by the University of Georgia, Athens Disease Diagnostic Laboratory (a member of NAHLN).

### **Polar Bear 1 (ID: 2023PB1007FDA; Collection date: 7-Oct-2023)**

An adult, male polar bear carcass in advanced decomposition was found; snout and claws were salvaged. Body condition was excellent. Because of advanced decomposition, only skin and skeletal muscle were collected for histopathologic examination. No evidence of inflammation, necrosis, or infectious agents was observed. Swab specimens (brain [spinal cord], rectal, nasal) tested negative for influenza virus by PCR.

### **Polar Bear 2 (ID: 2023PB1007FDB; Collection date: 7-Oct-2023)**

A subadult, male polar bear carcass in moderate decomposition was found. Body condition was good and no external injuries or gunshots were noted by the hunter. Brain (spinal cord) and skeletal muscle were collected from the salvaged head. Histopathologic examination showed a few myofibers contained nematodes (*Trichinella* spp.). No evidence of inflammation, necrosis, hemorrhage, vasculitis, or infectious agents was observed in the brain. Swab specimens (brain, nasal) tested negative for influenza virus by PCR.

### **Polar Bear 3 (ID: 2023PBRexford; Collection date: 25-August-2023)**

A subadult, male polar bear carcass in advanced decomposition was found. No external injuries or gunshots were noted by the hunter. Only skin and skeletal muscle were collected for histopathologic examination. No evidence of inflammation, necrosis, or infectious agents was observed. Swab specimens (brain, nasal) tested negative for influenza virus by PCR.

### **Polar Bear 4 (ID: 2023PB1204DL; Collection date: 4-Dec-2023)**

Polar bear 4, a subadult, male in excellent body condition, was alive and exhibited aggression toward vehicles. Deterrence efforts by North Slope Borough polar bear patrol staff

were unsuccessful, and the bear was killed by two gunshots to the head. Gross and histopathologic findings showed trauma to the costochondral joints (ribs 2–6) with focal muscle necrosis of the adjacent muscles, lipid pneumonia, hepatic and splenic pallor (hypovolemia), and a focal chronic renal infarct. Brain tissue could not be examined because of the kill method. Bird feathers with other food items (bowhead whale blubber and meat) were observed in the gastrointestinal tract. No infectious agents were observed in the lungs. Because of the bird feathers in the gastrointestinal tract and the aggressive antemortem behavior of the polar bear, influenza A virus immunohistochemistry was performed on formalin-fixed paraffin-embedded sections of lung; influenza A staining was negative.