DOI: 10.7589/JWD-D-20-00151

Journal of Wildlife Diseases, 57(3), 2021, pp. 652–656

A Novel Adenovirus Detected in Bering-Chukchi-Beaufort Seas Bowhead Whale (*Balaena mysticetus*): Epidemiologic Data and Phylogenetic Characterization

Eliana De Luca,[1,5] Raphaela Stimmelmayr,[2,3] David S. Rotstein,[4] and Susan Sanchez[1] [1]Athens Veterinary Diagnostic Laboratory, Department of Infectious Diseases, University of Georgia, 501 D. W. Brooks Drive, Athens, Georgia 30602, USA; [2]North Slope Department of Wildlife Management, 1274 Agvik Street, Utqiaġvik, Alaska 99723, USA; [3]Institute of Arctic Biology, University of Alaska Fairbanks, 902 N Koyukuk Drive, Fairbanks, Alaska 99775, USA; [4]Marine Mammal Pathology Services, 19117 Bloomfield Road, Olney, Maryland 20832, USA; [5]Corresponding author (email: elianadeluca9@gmail.com)

ABSTRACT: Adenoviruses are common pathogens infecting a wide range of vertebrates. Few cetacean adenoviruses have been described in the literature, and their pathogenicity is still unclear. Using PCR-based viral and bacterial pathogen surveillance in Bering-Chukchi-Beaufort seas bowhead whales (*Balaena mysticetus*) legally harvested 2012–15 during Alaskan aboriginal subsistence hunts, six of 59 bowhead whales (10%) tested positive for adenovirus DNA in the spleen. We found a high degree of sequence divergence from other mastadenoviruses, suggesting these may represent a novel species, tentatively named *bowhead whale adenovirus*. The sequences detected are distinct from adenoviruses previously identified in bottlenose dolphins (*Tursiops truncatus*) and harbor porpoises (*Phocoena phocoena*), forming two distinct clades in the cetacean hosts. The clinical impact is unclear, since no histopathologic evidence of adenovirus-associated disease was found. Furthermore, detection of adenovirus DNA in the spleen, contrary to other cetacean adenoviruses detected in the intestinal tract, may suggest a broader tissue tropism. Our study demonstrates adenovirus infection in bowhead whales and the usefulness of molecular diagnostics to discover and genetically characterize novel viruses in marine mammals.

Key words: Adenovirus, *Balaena mysticetus*, bowhead whales, marine mammals, PCR, phylogeny.

Today, as in the past, bowhead whales (*Balaena mysticetus*) constitute an essential nutritional, cultural, and spiritual subsistence resource for northern indigenous communities. Aboriginal bowhead whale hunting occurs during the spring and fall in 11 Alaskan whaling communities. This has been regulated by a quota system under the authority of the International Whaling Commission since 1977. Under the bowhead whale harvest monitoring program established in 1972 and collaboratively led by the North Slope Borough Department of Wildlife Management (NSB DWM) and the Alaska Eskimo Whaling Commission (AEWC), Bering-Chukchi-Beaufort seas bowheads harvested for subsistence undergo postmortem examination by hunters, biologists, and veterinarians to assess the health status of the landed whales and to collect tissue specimens and baseline data on life history, natural diseases, and marine threats. Although, historically, few infectious disease agents have been detected in bowhead whales, climate change has driven rapid Arctic and subarctic marine ecosystem transformations, which may alter host-pathogen interactions (Stimmelmayr et al. 2021).

From 2012–15, 59 landed bowhead whales had sex and age determined based on a combination of published criteria (George et al. 2018; O'Hara et al. 2002). Tissue samples (spleen, liver, kidney, lung, mesenteric lymph node, and skeletal muscle) from 59 bowhead whales were collected. Fresh tissues were stored at –80 C for viral molecular diagnostics or collected in 10% formalin for histopathologic evaluation. Formalin-fixed samples were processed routinely, sectioned at 5 μm, and stained with H&E stain.

Spleen, kidney, liver, and lung tissues were submitted to the Athens Veterinary Diagnostic Laboratory for molecular detection of 10 cetacean pathogens using PCR assays. All tissues were tested for the presence of morbilliviruses, whereas spleen samples were tested for the presence of *Brucella* spp. and *Salmonella* enterica (Baily et al. 1992; Daum

TABLE 1. Total body length (TBL) and demographics of the six adenovirus-positive bowhead whales (*Balaena mysticetus*) harvested 2012–15 during Alaskan aboriginal subsistence hunts. Whales were classified as sexually immature based on TBL (immature female, <13.7 m; immature male, <13 m; George et al. 2018; O'Hara et al. 2002). The sex of each whale was determined based on the length of the genital slit (longer in males [M] than in females [F]).

Animal ID	Year	Harvest season	Month	TBL (m)	Sex
12B8[a]	2012	Spring	April	8.3	M
12B22[a]	2012	Fall	October	9.19	F
13B15	2013	Fall	September	9.7	M
13B21	2013	Fall	October	8.58	F
14B14[a]	2014	Fall	October	10.05	F
15B19[a]	2015	Fall	October	8.15	M

[a] Bowhead whales from which tissues were evaluated by histopathology.

et al. 2002). Kidney and liver tissues were used for *Leptospira* spp. and vesiviruses PCR assays, respectively, whereas PCRs for paramyxoviruses, influenza A, *Cryptococcus neoformans*, *Mycoplasma* spp., vesiviruses, and coronaviruses were performed from lung tissues, following previously published protocols (Spackman et al. 2003; Tong et al. 2008).

To detect adenoviral DNA, a nested-PCR assay was performed on spleen samples using degenerate consensus primers amplifying a 318-base pair portion of the DNA polymerase (*pol*) gene sequence of adenoviruses (Wellehan et al. 2004). The identity of the amplified products was confirmed by Sanger sequencing. A *pol* gene nucleotide alignment with reference adenoviruses was employed in the phylogenetic analysis using MEGA X (Kumar et al. 2018).

Adenovirus-like particles have been isolated and observed by electron microscopy from the intestinal tissues or contents of a sei whale (*Balaenoptera borealis*) in the Antarctic (Smith and Skilling 1979), two bowhead whales in Alaska (Smith et al. 1987), and a beluga whale (*Delphinapterus leucas*) from the St. Lawrence estuary (De Guise et al. 1995). However, neither gross nor histopathologic lesions potentially associated with the infection were observed in any of those cases.

TABLE 2. Histopathologic findings in the six adenovirus positive bowhead whales (*Balaena mysticetus*). Whales were harvested 2012–15 during the Alaskan aboriginal subsistence hunts and underwent postmortem examination to assess their health status.[a]

Whale ID	Nonsplenic findings	Splenic findings
12B8	Renal intraductal mineral	NSF
12B22	Hepatic fibrosis with granuloma	NSF
13B15	NA	NA
13B21	NA	NA
14B14	Renal intraductal mineral	NSF
15B19	Renal interstitial fibrosis	NSF

[a] NA = not available; NSF = no significant histopathologic findings.

The only clearly established associations between adenovirus infection and disease in marine mammals have been found in California sea lions (*Zalophus californiasus*), showing gastroenteritis with severe hepatitis (Inoshima et al. 2013); in bottlenose dolphins (*Tursiops truncatus*), with mild gastroenteritis (Rubio-Guerri et al. 2015); and, more recently, in a captive polar bear (*Ursus maritimus*), with severe hepatic degeneration and necrosis (Dayaram et al. 2018). We present three adenovirus *pol* gene sequences identified from bowhead whales, which potentially represent a novel species of cetacean adenovirus, tentatively designated as *bowhead whale adenovirus* (BwAdV).

Six out of the 59 bowhead whales (10%; 95% confidence interval, 2.4–17.7) tested positive for adenovirus DNA from the spleen; demographics of the six positive animals are summarized in Table 1. No positive PCR results were obtained from tissues tested for other cetacean pathogens.

Tissues from four positive animals were examined histologically. Three whales had focal renal interstitial fibrosis (n=1) and small accumulations of minerals within the renal medullary ducts (n=2). Focal hepatic fibrosis with a granuloma was found in one whale. There were no splenic lesions, and splenic periarteriolar lymphoid sheaths were well-formed with moderate cellularity (Table 2). Intranuclear viral inclusion bodies were not

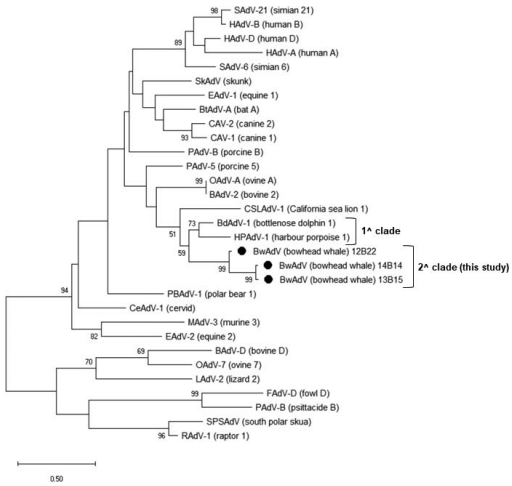

FIGURE 1. Evolutionary analysis of the adenovirus (AdV) sequences obtained from three of six adenovirus-positive bowhead whales (*Balaena mysticetus*) harvested 2012–15 during Alaskan aboriginal subsistence hunts. The evolutionary analysis was conducted in MEGA X (Kumar et al. 2018). The evolutionary history was inferred by the maximum-likelihood method and the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985). The tree with the highest log-likelihood value (–6,145.28) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying the neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and, then, selecting the topology with the superior log-likelihood value. A discrete γ-distribution was used to model evolutionary-rate differences among sites (five categories; +G, parameter = 0.4755). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 31 nucleotide sequences. The strains detected in our study are indicated by black circles. The following reference strains of the *Adenoviridae* family were included: 21 mastadenoviruses, two aviadenoviruses, three atadenoviruses, and two siadenoviruses. GenBank accession nos: human AdV A, AC_000005; human AdV B, KY320276; human AdV D, LC314153; simian AdV 6, JQ776547; simian AdV 21, AC_000010; murine AdV 3, NC_012584; porcine AdV B, MK774519; orcine AdV 5, NC_002702; sea lion AdV, KJ563221; bat AdV A, LC385828; equine AdV 1, KU133477; equine AdV 2, NC_027705; skunk AdV, NC_027708; bovine AdV 2, AF252854; ovine AdV A, AC_000001; polar bear AdV, MH115806; deer AdV 1, KY306667; canine AdV 1, AC_000003; canine AdV 2, AC_000020; bottlenose dolphin AdV 1, LT841149; harbor porpoise AdV, KY352473 (mastadenoviruses); fowl AdV, KU310942; psittacine AdV, NC_039032 (aviadenoviruses); ovine AdV 7, U40839; bovine AdV D, NC_002685; lizard AdV 2, NC_024684 (atadenoviruses); raptor AdV 1, NC_015455; south polar skua AdV, NC_016437 (siadenoviruses).

observed in the positive spleen tissues. Of the six tissue samples positive for adenovirus DNA, three yielded DNA suitable for Sanger sequencing. Three 290-base pair *pol* gene sequences were obtained and deposited in GenBank (accession nos. MT461296, MT461297, and MT461298).

Our study's sequences shared from 62% to 74.8% of nucleotide (nt) identity and from 62% to 82.98% of amino acid (aa) identity with the 21-reference strains of mastadenoviruses included in the analysis. The highest nt sequence identity was shared with bottlenose dolphin adenovirus 1, BdAdV-1 (65.6–72.3% of nt identity), followed by bovine adenovirus 2, BAdV-2 (67.7–70.5% of nt identity), harbor porpoise adenovirus 1, HpAdV-1 (64.1–70.2% of nt identity), porcine mastadenovirus B, PAdV-B (63.8–67.7% of nt identity), and California sea lion adenovirus 1, CSLAdV-1 (62–62.7% of nt identity). In addition, our sequences were not identical to each other, sharing an nt identity ranging from 87.2% to 96% and an aa identity ranging from 88.3% to 96.8%. Within the *Adenoviridae* family, differences greater than 15% in the DNA polymerase aa sequence are consistent with distinct species, whereas aa differences less than 15% are seen between distinct types of a species (Harrach et al. 2011). Therefore, it appears likely that our sequences represent a novel species within the *Adenoviridae* family and that the strains may be classified as two distinct subtypes. Phylogenetic analysis confirmed that the sequences belong to the *Mastadenovirus* genus, grouped together in a separate clade supported with a 99% bootstrap value (Fig. 1). According to the phylogenetic tree, they were distantly related to adenoviruses previously identified in other cetaceans, indicating the presence of two different clades infecting cetaceans, the first, including BdAdV-1 and HpAdV-1, whereas the second clade includes the sequences from this study.

Prevalence comparison with previous studies on bowhead whales is difficult given the variety of diagnostic tests used and the different number of animals enrolled. The prevalence in this study (10%; 6/59) is lower

than that previously reported for Bering-Chukchi-Beaufort seas bowhead whales (50%; 2/4; Smith et al. 1987). Similarity searches against adenoviruses sequences publicly available revealed that the most closely related viruses were BdAdV-1 and HpAdV-1, followed by bovine and porcine adenoviruses. This is in line with what has already been described for BdAdV-1 (Malmberg et al. 2017), which is most closely related to bovine and porcine adenoviruses and more distantly related to adenoviruses of other aquatic mammals, such as CSLAdV-1. Within members of the *Adenoviridae* family, greater guanine-cytosine (G/C) content and lower pathogenicity are associated with longstanding host-virus relationships, whereas lower G/C content is related to recent host jumps (Wellehan et al. 2004). The G/C-content of sequences in this study was higher (48.2–52.1%) than CSLAdV-1 (36%) and BdAdV-1 (36.2%), which are both associated with disease in their respective hosts (Inoshima et al. 2013; Malmberg et al. 2017). Although our data are limited to a partial fragment of the *pol* gene, our findings suggest that BwAdV may be a bowhead whale–specific virus that has been co-evolving with its host.

The detection of BwAdV in splenic tissue from individual bowhead whales harvested in different years (Table 1) further supports the idea that bowhead whales represent the natural hosts of this virus. In addition, no histopathologic evidence of adenovirus-associated disease was found in the six whales. The absence of viral inclusions could reflect a mechanism of viral latency, which has been extensively reported for human adenoviruses (Zhang et al. 2010). It is possible that BwAdV is a common infection and that disease only develops if predisposing factors are present. Future studies may permit assessment of the real prevalence of adenovirus infection among the whale population. We detected BwAdV in lymphoid tissues, which contrasts with the other cetacean adenoviruses identified from intestinal and hepatic tissues. Whether this phenomenon is related to a broader tissue tropism should be investigated. In conclusion, our study shows that adenovirus infection occurs in bowhead whales and highlights the role of advanced biotechnologies in discovering and characterizing new viruses in marine mammals. Further studies are needed to fully characterize the viral genome, explore the tissue tropism, and define the phylogeny among adenovirus strains in bowhead whales.

We thank the Barrow Whaling Captains Association and the AEWC for allowing us to examine their landed bowhead whales and to conduct the study. This study was funded by qualified outer continental shelf oil and gas revenues by a substantial grant from the Coastal Impact Assistance program, the US Fish and Wildlife Service, the US Department of the Interior, and the NSB DWM. Collection of marine mammal tissues was conducted under NOAA-NMFS permit 17350–01. We would like also to acknowledge the staff of the molecular section of the Athens Veterinary Diagnostic Laboratory, Athens, Georgia, US. E.D.L. was supported by the College of Veterinary Medicine at the University of Georgia and Boehringer Ingelheim.

LITERATURE CITED

Baily GG, Krahn JB, Drasar BS, Stoker NG. 1992. Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. *J Trop Med Hyg* 95:271–275.

Daum LT, Barnes WJ, McAvin JC, Neidert MS, Cooper LA, Huff WB, Gaul L, Riggins WS, Morris S, Salmen A, et al. 2002. Real-time PCR detection of *Salmonella* in suspect foods from a gastroenteritis outbreak in Kerr County, Texas. *J Clin Microbiol* 40:3050–3052.

Dayaram A, Tsangaras K, Pavulraj S, Azab W, Groenke N, Wibbelt G, Sicks F, Osterrieder N, Greenwood AD. 2018. Novel divergent polar bear-associated mastadenovirus recovered from a deceased juvenile polar bear (*Ursus maritimus*). *mSphere* 3:e00171-18.

De Guise S, Lagacé A, Béland P, Girard C, Higgins R. 1995. Non-neoplastic lesions in beluga whales (*Delphinapterus leucas*) and other marine mammals from the St Lawrence Estuary. *J Comp Pathol* 112: 257–271.

George JC, Suydam R, Givens G, Horstmann L, Stimmelmayr R, Sheffield G. 2018. *Length at sexual maturity and pregnancy rates of Bering-Chukchi-Beaufort seas bowhead whales* (Balaena mysticetus). Report SC 67b/AWMP/07 presented to the International Whaling Commission. https://archive.iwc.int/pages/search.php?search=!collection29526&bc_from=themes. Accessed February 2021.

Harrach B, Benko M, Both GW, Brown M, Davison A, Echavarrı AM, Hess M, Jones MS, Kajon A,

Lehmkuhl HD. 2011. Family *Adenoviridae*. In: *Virus taxonomy (9th report of the International Committee on Taxonomy of Viruses)*, King AMW, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Elsevier, Waltham, Massachusetts, pp. 125–141.

Hasegawa M, Kishino H, Yano T. 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. *J Mol Evol* 22:160–174.

Inoshima Y, Murakami T, Ishiguro N, Hasegawa K, Kasamatsu M. 2013. An outbreak of lethal adenovirus infection among different otariid species. *Vet Microbiol* 165:455–459.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35:1547–1549.

Malmberg M, Rubio-Guerri C, Hayer J, García-Párraga D, Nieto-Pelegrín E, Melero M, Álvaro T, Valls M, Sánchez-Vizcaíno JM, Belák S, et al. 2017. Phylogenomic analysis of the complete sequence of a gastroenteritis-associated cetacean adenovirus reveals a high degree of genetic divergence. *Infect Genet Evol* 53:47–55.

O'Hara TM, George JC, Tarpley RJ, Burek J, Suydam RS. 2002. Sexual maturation in male bowhead whales (*Balaena mysticetus*) of the Bering seas stock. *J Cetacean Res Manag* 4:143–148.

Rubio-Guerri C, García-Párraga D, Nieto-Pelegrín E, Melero M, Álvaro T, Valls M, Crespo JL, Sánchez-Vizcaíno JM. 2015. Novel adenovirus detected in captive bottlenose dolphins (*Tursiops truncates*) suffering from self-limiting gastroenteritis. *BMC Vet Res* 11:53.

Smith AW, Skilling DE. 1979. Viruses and virus diseases of marine mammals. *J Am Vet Med Assoc* 175:918–920.

Smith AW, Skilling DE, Benirschke K, Albert TF, Barlough JE. 1987. Serology and virology of the bowhead whale (*Balaena mysticetus*). *J Wildl Dis* 23: 92–98.

Spackman E, Senne DA, Bulaga LL, Myers TJ, Perdue ML, Garber LP, Lohman K, Daum LT, Suarez DL. 2003. Development of real-time RT-PCR for the detection of avian influenza virus. *Avian Dis* 47 (Suppl 3):1079–1082.

Stimmelmayr R, Rotstein D, Sheffield G, Brower H, George J. 2021. Diseases and parasites. In: *The Bowhead whale—Balaena mysticetus: Biology and human interactions*, George JC, Thewissen JGM, editors. Academic Press, London, UK, pp. 471–498.

Tong S, Chern SW, Li Y, Pallansch MA, Anderson LJ. 2008. Sensitive and broadly reactive reverse transcription-PCR assays to detect novel paramyxoviruses. *J Clin Microbiol* 46:2652–2658.

Wellehan JFX, Johnson AJ, Harrach B, Benkö M, Pessier AP, Johnson CM, Garner MM, Childress A, Jacobson ER. 2004. Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. *J Virol* 78:13366–13369.

Zhang Y, Huang W, Ornelles DA, Gooding LR. 2010. Modeling adenovirus latency in human lymphocyte cell lines. *J Virol* 84:8799–8810.

Submitted for publication 19 August 2020.
Accepted 17 December 2020.