

Detection of Bisgaard Taxon 40 in Rhinoceros Auklets (*Cerorhinca monocerata*) with Pneumonia and Septicemia from a Mortality Event in Washington, USA

Susan Knowles,^{1,4} Barbara L. Bodenstein,¹ Brenda M. Berlowski-Zier,¹ Susan M. Thomas,² Scott F. Pearson,³ and Jeffrey M. Lorch¹ ¹US Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, USA; ²Washington Maritime National Wildlife Refuge Complex, US Fish and Wildlife Service, 715 Holgerson Road, Sequim, Washington 98382, USA; ³Wildlife Science Division, Washington Department of Fish and Wildlife, 1111 Washington Street SE, Olympia, Washington 98501, USA; ⁴Corresponding author (email: sknowles@usgs.gov)

ABSTRACT: We isolated Bisgaard taxon 40 from Rhinoceros Auklets (*Cerorhinca monocerata*) with pneumonia and septicemia from Washington, US, found dead in 2016. Previously isolated only from the respiratory tract of a gull (Laridae), little is known about its pathogenic potential and whether it acts as a primary or opportunistic pathogen.

Rhinoceros Auklets (*Cerorhinca monocerata*) are seabirds in the order Charadriiformes and family Alcidae and are the only living member of the genus *Cerorhinca*. They are distributed in the Pacific Ocean, with their breeding range extending from the Aleutian Islands and Gulf of Alaska south to California and Japan (Nettleship 1996). In the US, large breeding colonies occur in Washington state on Protection and Destruction Islands and in Southeast Alaska on Forrester Island (Gaston and Dechesne 1996).

In spring and summer 2016, approximately 300 dead Rhinoceros Auklets washed ashore in the eastern part of the Strait of Juan de Fuca in Washington state. During this time, shellfish harvesting in the area was closed because of the detection of saxitoxin, a potent neurotoxin that causes paralytic shellfish poisoning. In June, a visitor at the Dungeness National Wildlife Refuge (48°10'N, 123°8'W) reported seeing birds “acting woozy.” Subsequently, refuge staff found seven dead Rhinoceros Auklets and submitted them to the US Geological Survey’s National Wildlife Health Center (Madison, Wisconsin, USA) for postmortem examination. In late July, a volunteer for the Coastal Observation and Seabird Survey Team found additional dead Rhinoceros Auklets at Lopez Island (48°26'N,

122°54'W) and submitted three carcasses for postmortem examination.

We performed postmortem examinations on three male and four female adult auklets. Six birds were emaciated, and one bird was in poor body condition. Significant gross findings included pneumonia with red to dark red, firm to meaty or rubbery lungs that often sank in formalin, suggesting decreased air or increased density (Fig. 1A). One bird had gross evidence of pleural involvement with a focally extensive area of thickened, yellow pleura (Fig. 1A). In the same bird, a 3.7×2.1-cm focus of pallor was noted in the left superficial pectoral muscle (Fig. 1B).

Examination of H&E-stained lung sections showed diffuse vascular congestion and arterial walls expanded by necrotic debris, fibrin and edema, heterophils and macrophages, and clusters of bacteria (6/6) that often extended into the surrounding air capillaries (Fig. 1C, D). A Brown and Hopps Gram stain revealed numerous gram-negative bacteria (Fig. 1C inset). A similar infiltrate with bacteria filled the lumen of occasional parabronchi (Fig. 1E). Bacteria were occasionally present in the wall of secondary bronchioles. The pleura (5/6) was mildly to greatly thickened by a layer of fibrin and edema admixed with abundant eosinophilic necrotic debris, degenerate heterophils, fewer macrophages, and gram-negative bacteria (Fig. 1F). In a single bird, the tracheal adventitia was greatly expanded by an infiltrate, as observed in the pleura (Fig. 1G). In another bird, a focally extensive area of the esophageal muscularis was replaced by eosinophilic necrotic debris with numerous bacterial clusters (Fig. 1H). Eosinophilic necrotic

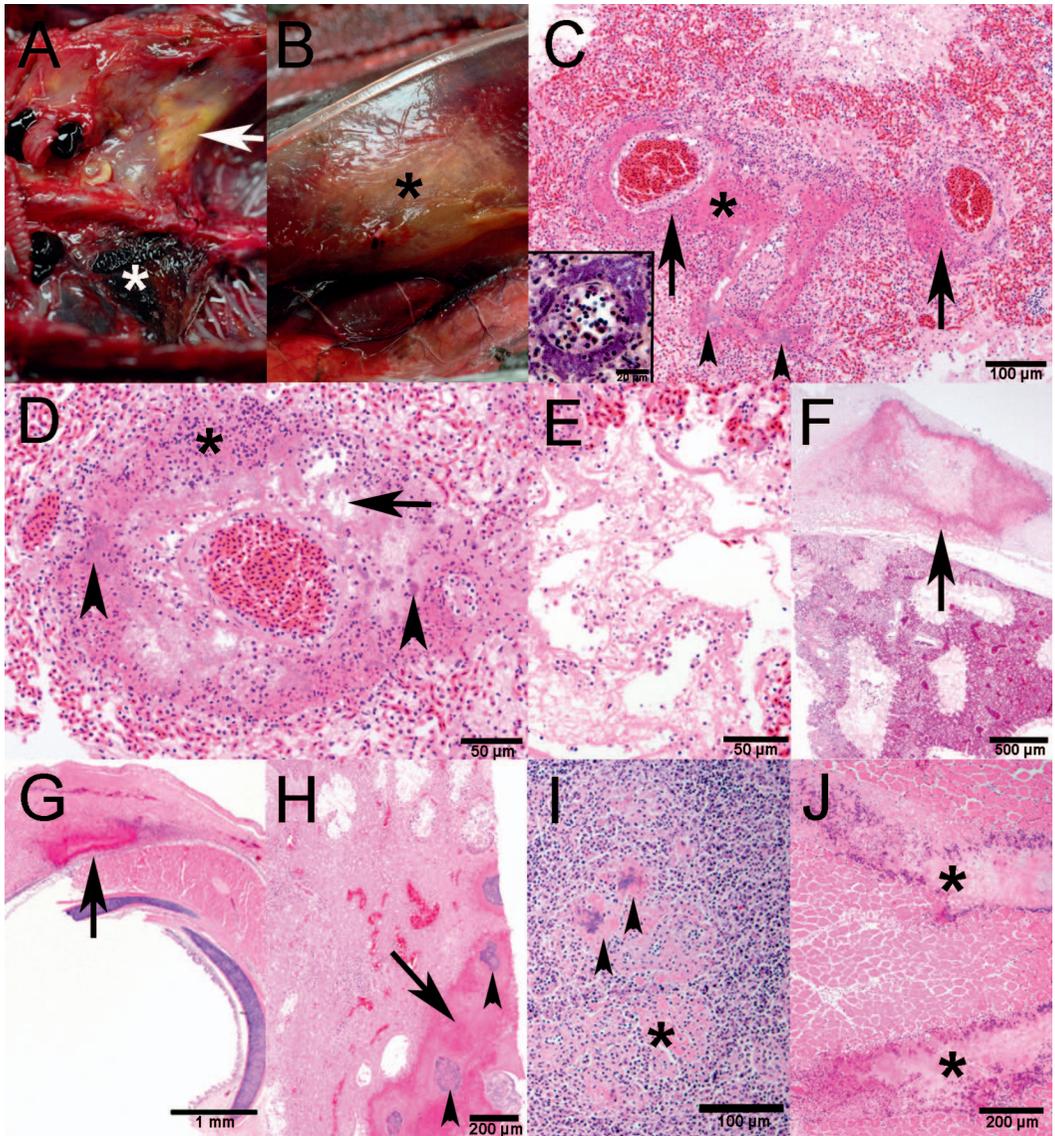


FIGURE 1. Gross photographs and photomicrographs (H&E stain) of Rhinoceros Auklets (*Cerorhinca monocerata*) found dead in Dungeness National Wildlife Refuge in Clallam County, Washington, USA. (A) Diffusely dark red pneumonic lung (*) and yellow thickened pleura (arrow). (B) A focally extensive area of pallor in the pectoral muscle (*). (C) Pulmonary vasculature is diffusely congested. Pulmonary arterial walls (arrows) are expanded by eosinophilic necrotic debris and inflammatory cells, fibrin and edema, and clusters of bacteria (arrowheads) that extend into the adjacent air capillaries (*). Inset: Arterial wall expanded by numerous gram-negative bacteria. Brown and Hopps Gram stain. (D) Higher magnification of pulmonary artery showing necrotic debris and degenerate inflammatory cells (*), fibrin and edema (arrow), and bacteria (arrowheads). (E) An inflammatory infiltrate as seen in pulmonary arteries fills the lumen of a parabronchus. (F) The pleura (arrow) is greatly expanded by a layer of fibrin and edema admixed with abundant eosinophilic necrotic debris, degenerate inflammatory cells, and bacteria. (G) The tracheal adventitia (arrow) is greatly expanded by a thick layer of edema and fibrin admixed with eosinophilic necrotic debris, inflammatory cells, and bacteria. (H) A focally extensive area of the esophageal muscularis is replaced by eosinophilic necrotic debris (arrow) with numerous bacterial clusters (arrowheads). (I) Splenic ellipsoids are filled with eosinophilic necrotic debris and fibrin (*) and clusters of bacteria (arrowheads). (J) Pectoral muscle fascial planes (*) are greatly expanded by fibrin and edema admixed with degenerate inflammatory cells, eosinophilic necrotic debris, and bacteria.

TABLE 1. Diagnostic summary of adult Rhinoceros Auklets (*Cerorhinca monocerata*) found dead on Dungeness National Wildlife Refuge and Lopez Island, Washington, USA, June–July 2016.

Location	Date	Sex ^a	Postmortem condition ^b	Significant histopathologic findings ^c	Bisgaard isolate–organ ^c
Dungeness National Wildlife Refuge	14 June	F	Good	Fibrinonecrotic pleuropneumonia, splenitis, cardiomyofiber necrosis	Bisgaard taxon 40–lung NA–liver
	14 June	M	Fair	Fibrinonecrotic pleuropneumonia, splenitis, esophagitis	Bisgaard taxon 40–lung NA–liver NA–spleen
	14 June	M	Good	Fibrinous pleuropneumonia, splenitis, tracheitis	Bisgaard taxon 40–liver
	20 June	F	Fair	Fibrinous pleuropneumonia, airsaccutitis, myositis	Bisgaard taxon 40–lung Bisgaard taxon 40–liver
Lopez Island	28 July	M	Fair	Fibrinonecrotic pleuropneumonia, splenitis	Bisgaard taxon 40–lung NA–liver NA–brain
	28 July	F	Fair	Necrotizing pneumonia, splenitis	Bisgaard taxon 40–lung Bisgaard taxon 40–liver Bisgaard taxon 40–brain
		F	Poor	NA	NA–lung Bisgaard taxon 32–liver Bisgaard taxon 32–brain

^a F = female; M = male.

^b Qualitative assessment of autolysis based on color, odor, gas distention, sloughing of skin, sunken eyes, intactness, and insect activity.

^c NA = not applicable.

debris and fibrinous material that was positive for fibrin with a phosphotungstic acid hematoxylin stain filled splenic ellipsoids (5/5), which often contained clusters of bacteria (Fig. 1I). In the pectoral muscle with the grossly observed pallor, fascial planes were greatly expanded by fibrin and edema admixed with degenerate heterophils, eosinophilic debris, and gram-negative bacteria (Fig. 1J). Adjacent myofibers were often necrotic. Other findings included bacterial airsaccutitis (1/6), splenic lymphoid depletion (5/5), focal cardiomyofiber necrosis with intralesional bacteria (1/6), renal inflammation (4/6), renal oocysts (2/6), intestinal cestodes (2/5), and sarcocysts in skeletal muscle (1/6).

We performed routine bacterial cultures on selected tissues (Table 1) using tryptic soy agar plates containing 5% sheep blood incubated at 37 C and identified bacterial isolates using biochemical tests (API strips, BioMerieux, Marcy l'Etoile, France; OmniLog microbial identification system, Biolog Inc., Hayward, California, USA) or by sequencing

an approximately 520-nucleotide (nt) portion (containing the V1–V3 regions) of the 16S ribosomal (r)RNA gene as described in Shearn-Bochsler et al. (2018). Bisgaard taxon 40 (100% sequence identity to GenBank no. AY172732 across a 496-nt portion of the 16S rRNA gene; a new representative sequence deposited as GenBank no. MG735704) was cultured from the lung of six auklets and the liver or brain of three birds. A second Bisgaard taxon most closely matching existing sequences in GenBank for Bisgaard taxa 14 and 32 (96% and 96–97% sequence identities for Bisgaard taxa 14 and 32, respectively, across a 390-nt portion of the 16S rRNA gene; new sequence deposited as GenBank no. MG735705) was cultured from the liver and brain of one auklet. Other isolated bacteria were normal avian gastrointestinal tract flora and likely represented postmortem colonizers. Tracheal and cloacal swabs were negative for avian influenza virus by reverse transcription PCR (Spackman et al. 2002), and brain tissue from Lopez Island birds was negative for West

Nile virus by culture (Docherty et al. 2004). Gastrointestinal tract contents had below detectable limits of saxitoxin ($n=7$) and domoic acid ($n=3$) based on enzyme-linked immunosorbent assay (Abraxis Inc., Warminster, Pennsylvania, USA) performed at the National Oceanic and Atmospheric Administration's Wildlife Algal-Toxin Research and Response Network for the US West Coast (Seattle, Washington, USA).

Members of *Pasteurellaceae* are often isolated from the respiratory, reproductive, and alimentary tracts of vertebrates, and, with few exceptions, are generally thought to be opportunistic invaders that typically coexist with the host (Christensen et al. 2003). Bisgaard taxa 40, 14, and 32 are members of the family *Pasteurellaceae* and form a clade with *Pasteurella testudinis* and *Chelonobacter oris* on the basis of 16S rDNA sequence analyses (Christensen et al. 2003; Gregersen et al. 2009). Bisgaard taxon 40 has only been isolated from the respiratory tract of a gull (Christensen et al. 2003), and taxa 14 and 32 only from a pheasant with septicemia and hawks with pneumonia and conjunctivitis, respectively (Bisgaard and Muters 1986; Bisgaard 1993; Christensen et al. 2003). Because of the poor postmortem state, histopathology was not performed on the bird from which an unidentified Bisgaard taxon was isolated. For that individual, it was difficult to ascertain whether the unidentified Bisgaard taxon was the etiologic agent or whether Bisgaard taxon 40 was not isolated because of the abundance of potential post-mortem colonizing bacteria. Bisgaard taxon 40 is a slow-growing bacterium that can easily be outcompeted by other bacteria in contaminated samples. The auklets with Bisgaard taxon 40 had gross and histologic evidence of pneumonia and septicemia. We do not know whether Bisgaard taxon 40 acted as a primary pathogen or, given the emaciated body condition of these birds, simply as an opportunistic pathogen. Additional research is necessary to study the disease potential of this pathogen.

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