Baseline Histological Health Assessment of Subsistence Harvested Arctic Marine Mammals from the North Slope Borough Village of Wainwright, Alaska

Greta M. Krafsur
319 Diagnostic Medicine Center
Colorado State University
North Slope Borough Department of Wildlife Management
PO Box 69
Barrow, AK 99723
klondikekrafsur@gmail.com

Terry R. Spraker
319 Diagnostic Medicine Center
Colorado State University
(970)-297-4155
Terry.Spraker@colostate.edu

Billy Adams
North Slope Borough Department of Wildlife Management
PO Box 69
Barrow, AK 99723

The author wishes to acknowledge the overwhelming kindness and generosity extended to her by members of the Wainwright community and to Ida Panik, Wainwright NSB DWM representative for her assistance. The author is especially grateful to the Wainwright subsistence hunters for kindly letting a stranger collect samples from their seals and walruses. Quyanaqpak!

Abstract: Tissue samples from subsistence harvested bearded seals (*Erignathus barbatus*) and walruses (*Odobenus rosmarus divergens*) were collected in the coastal village of Wainwright, Alaska in June 2009. Tissues were collected from 12 bearded seals and 5 walruses for routine histological examination. One seal had pyogranulomatous panniculitis, likely caused by a bite wound. Exertional rhabdomyolysis was a common histological finding in seven of twelve seals and three of five walruses. One tissue cyst resembling *T*. gondii was identified in the tongue skeletal muscle of one seal. Sarcocysts were identified in the skeletal muscles of eight of twelve bearded seals, but not in walrus. There were no histopathological renal lesions suggestive of *Leptospira spp* infection. Loss of sea ice, warmer temperatures, increased ship traffic and oil and gas activity make disease surveillance of marine mammal populations that are important subsistence resources for Alaska's indigenous coastal populations a high priority for scientists working with the North Slope Department of Wildlife Management in Barrow, Alaska.

Introduction

Marine mammal subsistence hunting has sustained the North Slope Iñupiat Eskimoes for thousands of years. The native culture stresses the importance of maintaining spiritual ties with the environment, respect for the animals that feed them and sharing with the community. In addition, the expenses associated with the importation of nontraditional foods make subsistence hunting a necessity. The bowhead whale (*Balaena mysticetus*) is the largest part of the Iñupiat Eskimo diet in Arctic coastal villages such as Barrow and Wainwright, Alaska. The ringed seal (*Phoca largha*), bearded seal (*Erignathus barbatus*) and walrus (*Odobenus rosmarus divergens*) are also important components of the subsistence diet. In addition, bearded seal skins are used for making the traditional skin-covered boat, the umiak, used during spring whaling. Skins from bearded and ringed seals are used for clothing and accessories while bones from the seal and walrus carcasses are used for making decorative art.

The people of the North Slope are very concerned about the preservation of their cultural traditions and the future availability of subsistence resources. Diminishing sea ice, increased ship traffic and expanding oil and gas development in the Chukchi and Beaufort Seas have the potential to devastate habitat critical to the survival of the animals that feed and clothe them. Warmer temperatures may permit the introduction and establishment of zoonotic pathogens such as *Brucella*, *Leptospira*, *Influenza A*, *Toxoplasma*, *Giardia*, *Cryptosporidia* and *Trichinella* in the North Slope wildlife populations, presenting risks to the animals and the hunters. Scientists with the North Slope Borough Department of Wildlife Management in Barrow, Alaska are working closely with community members from the North Slope villages to monitor the health of subsistence resources with the intent of generating baseline datasets from areas not yet impacted by industrial development. Tissues have been collected from healthy hunter-harvested animals and those animals with gross lesions that are unfit for consumption. Departmental scientists have examined carcasses, taken morphological measurements, collected teeth for aging and examined stomach contents in addition to collecting a full complement of tissues for disease surveillance, contaminants analysis and histological studies. Tissues collected are of the highest quality because scientists generally accompany the hunters and are able to collect them from freshly dead animals. In addition to establishing baseline values for the health of these animals, these tissue collections represent a rare and unparalled opportunity to gain valuable insight about the structure-function relationship in marine mammal anatomy and physiology and the histological attributes of marine mammal tissues.

Materials and Methods

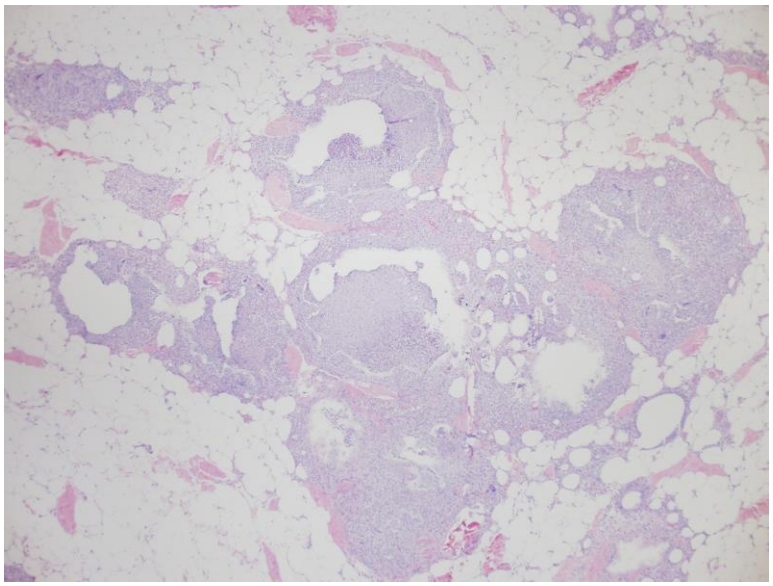
Twelve hunter-harvested bearded seals and five walruses taken near the coastal village of Wainwright, Alaska (70˚ 38 ‘ 50’’ N 160˚ 0’ 58” W) were sampled by a North Slope Borough scientist during the summer 2009 marine mammal harvest. Wainwright is 72 miles southwest of Barrow, its coastline is bordered by the Chukchi Sea. Approximately 500 people live in the coastal village, 90% are Iñupiat Eskimo. Community members were informed by VH radio that tissue collection would take place, hunters either submitted tissues or contacted the scientist when harvesting was ready to commence. Alaska’s indigenous peoples are permitted to hunt marine mammals according to guidelines set forth by the 1972 Marine Mammals Protection Act. The North Slope Borough Department of Wildlife Management collects tissues for scientific studies from animals with the permission of Marine Mammal Permits **(NMFS permit #814-1899-00, seals)** and **(NMFS permit #MA134907, walruses)**.

Carcasses were examined where possible and information concerning age, gender, time of death, and time of tissue collection and subsampling were noted, in addition to hunter contact information. The presence of gross lesions was noted and described. Tissues were preserved in 10% neutral buffered formalin, similar tissues from the same organs were frozen for future diagnostic tests. The formalin-fixed tissues were trimmed at the North Slope Borough Department of Wildlife Management Lab in Barrow, Alaska and brought to the Colorado State University Veterinary Medical Diagnostics Center in Fort Collins, Colorado in January 2010. Tissue blocks were paraffin-embedded, sectioned and stained with Mayer’s hematoxylin and eosin (H & E).

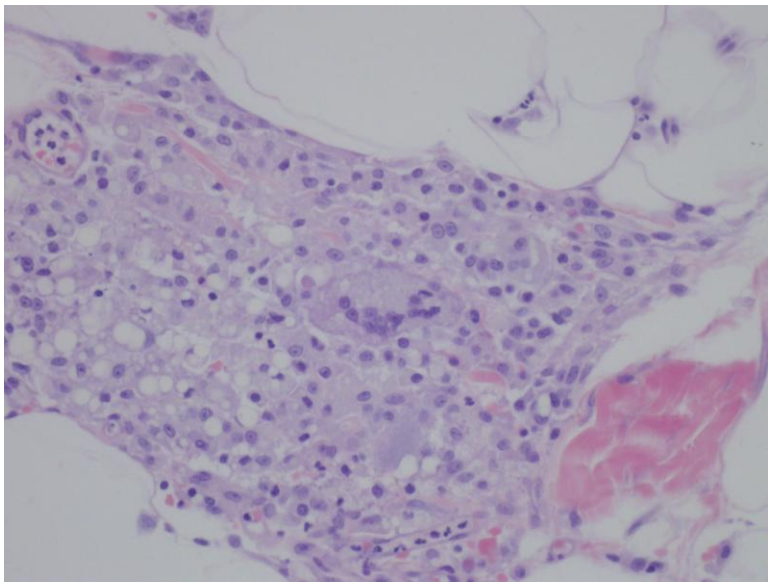
Results

One seal had scarring of the skin proximal to the dorsal surface of the pelvic flippers. Deep to these scars were multifocal pyogranulomatous lesions that were located within the entire blubber layer. Pyogranulomatous panniculitis with histological evidence of chronic hemorrhage and hemosiderin, suppurative inflammation, and multinucleate giant cells were found in the lesions. These lesions were consistent with bite wounds and introduction of

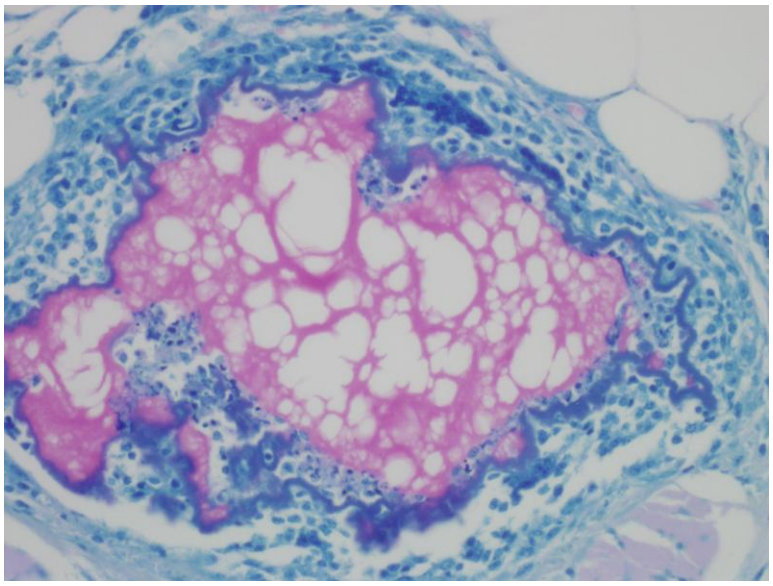
microorganisms or a foreign body. Acid fast and Gomori-Grocott Methenamine Silver stains failed to demonstrate positive staining for microorganisms, however.



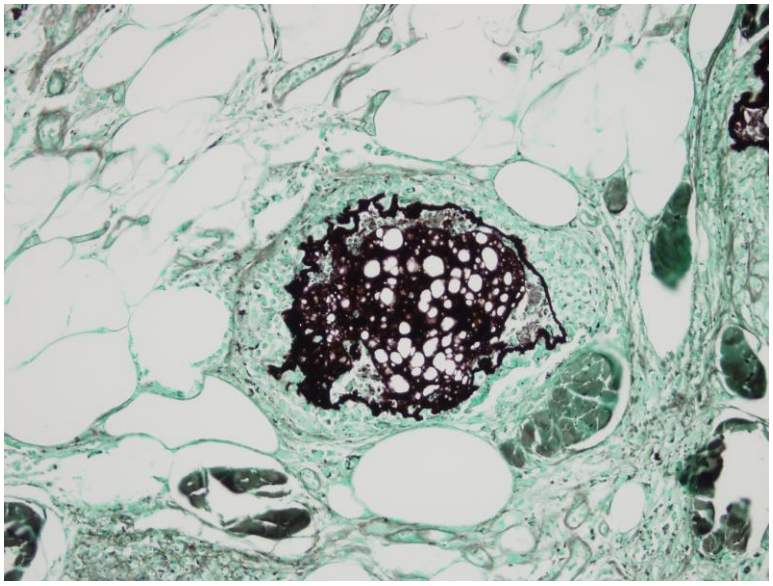
O9WWBS7 Blubber 4x



O9WWBS7 Blubber 40x, Multinucleate Giant Cell with Cellular Debris

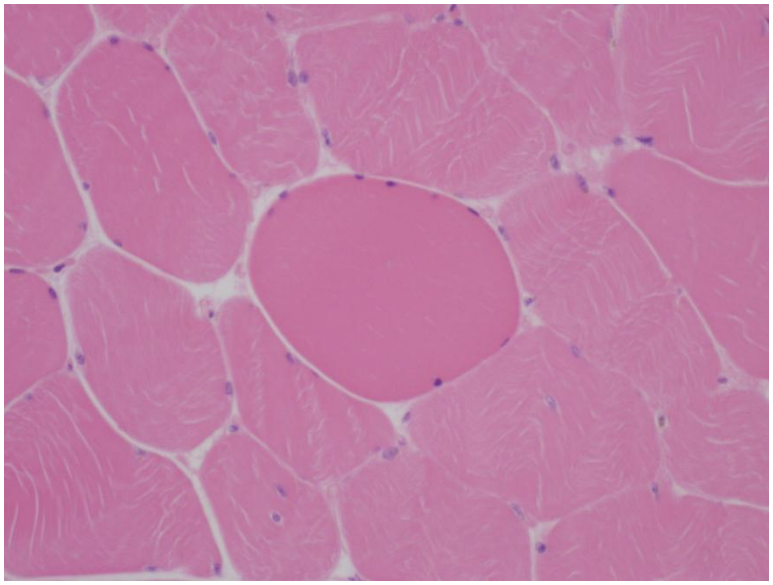


09WWBS7 Blubber 40x, Acid Fast Stain

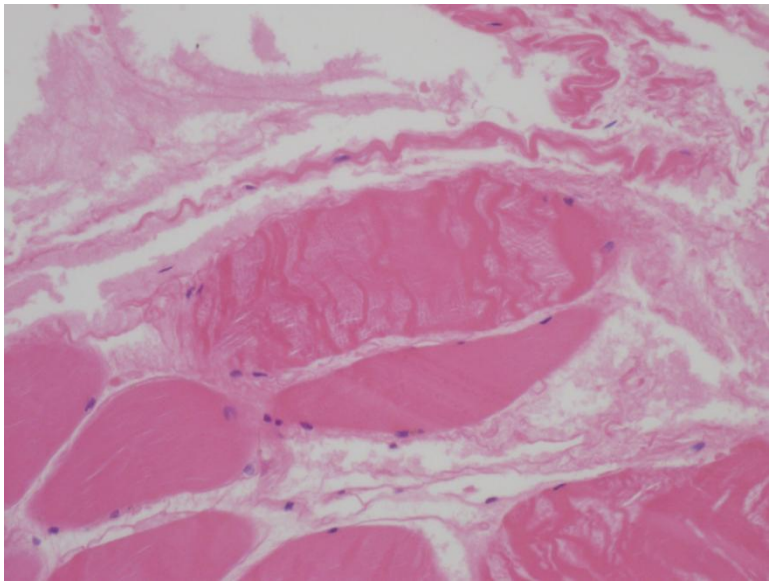


09WWBS7 Blubber 40x, GMS Stain

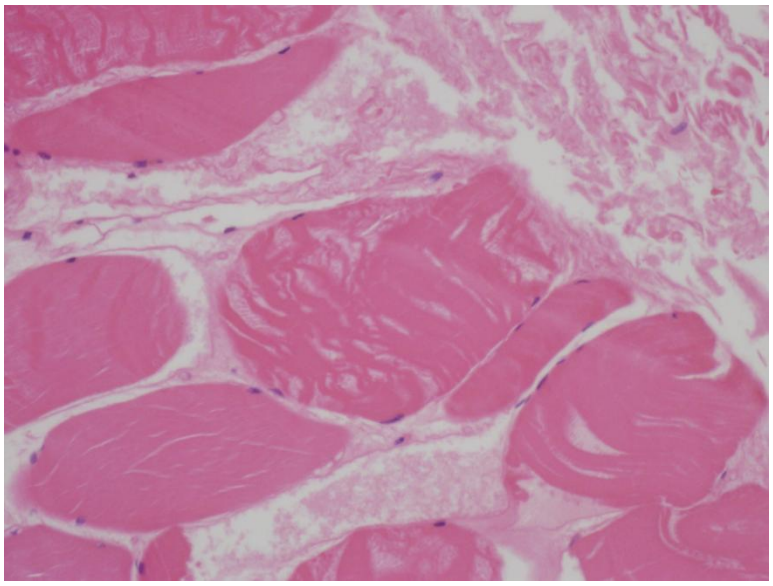
The various stages of myositis and rhabdomyolysis were consistently observed in skeletal muscle sections from seven of twelve bearded seals and three of five walruses. Early swelling, fragmentation and cleavage of sarcoplasma, inflammation and healing were all observed.



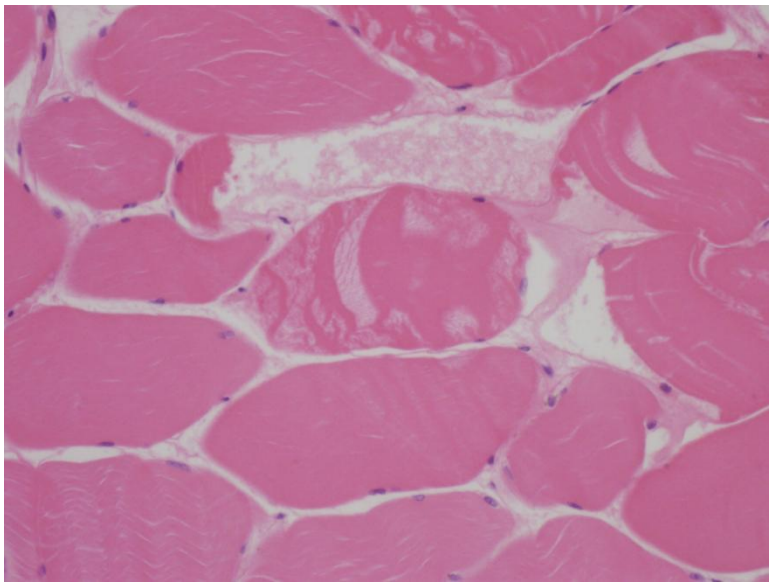
09WWBS11 Skeletal Muscle 40x, Early Cell Swelling



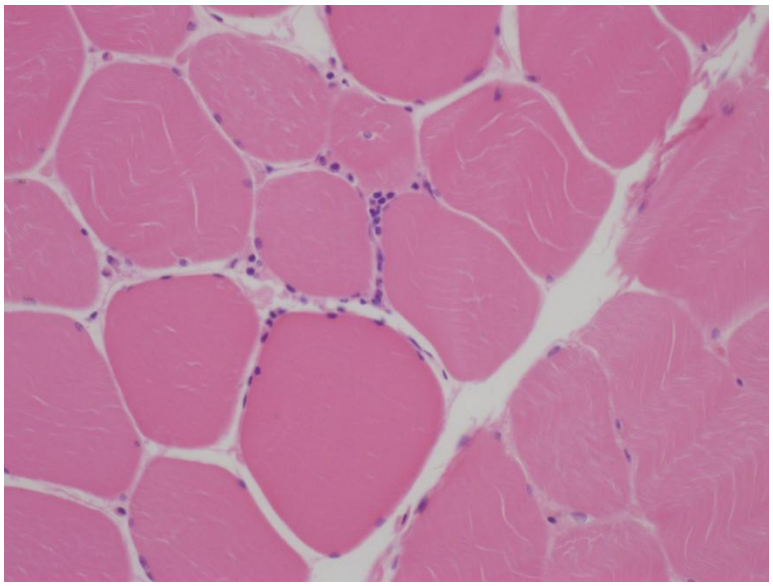
09WWBS9 Skeletal Muscle 40x, Fragmentation Cleavage



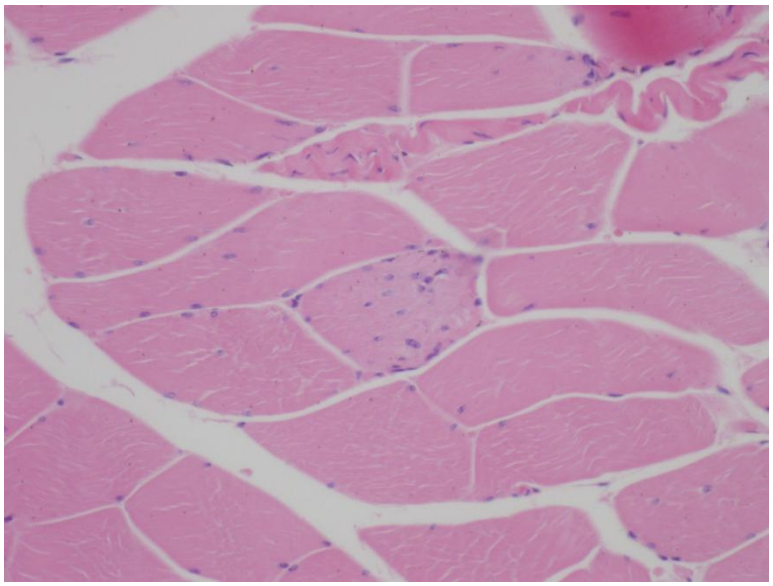
09WWBS9 Skeletal Muscle 40x, Fragmentation Cleavage



09WWBS9 Skeletal Muscle 40x, Fragmentation Cleavage

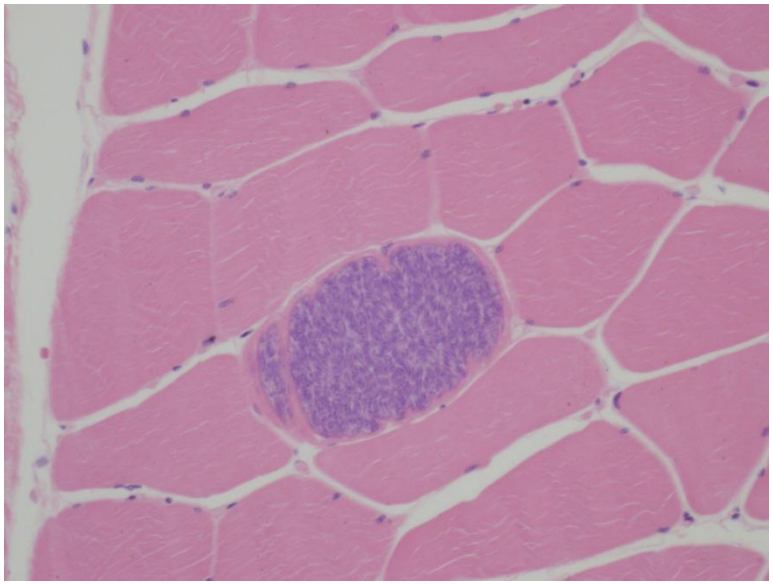


09WWBS9 Skeletal Muscle 40x, Inflammation

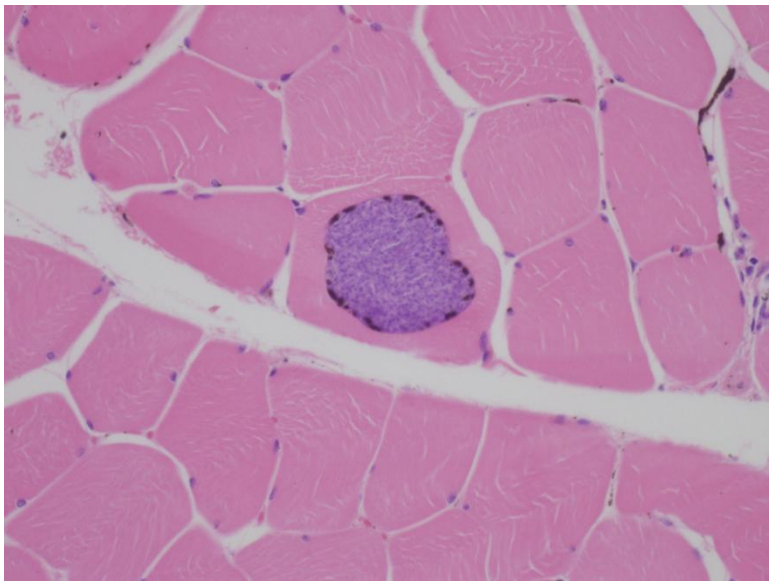


09WWBS9 Skeletal Muscle 40x, Myocyte Healing

Tissues cysts suggestive of *Sarcocystis neurona*-like infection were identified in skeletal muscle sections from eight of the twelve bearded seals but not in walrus skeletal muscles. No local tissue inflammatory reactions were observed. Based on histological appearance of the tissue cysts, the researcher believes these particular sarcocysts are unlike others previously described in the marine mammal parasitology literature. A tissue cyst with small bradyzoites was found in a section of tongue that was different than others found in the skeletal muscle, suggestive of *Toxoplasma gondii*.



09WWBS11 Skeletal Muscle, 40x Sarcocyst



09WWBS11 Skeletal Muscle 40x, Mature Sarcocyst

These animals were in good body condition with abundant fat around the coronary vasculature. A small fibromuscular intimal plaque was noted in one of the coronary arteries from a walrus. A single focus of mild degenerative myocarditis was noted in another walrus. Lung parenchyma was within normal limits. The seal with pyogranulomatous panniculitis had lungs with histological evidence of a mild multifocal pneumonia.

Tonsils, lymph nodes, thymus and spleen also were examined. The lymph nodes were mostly active and within normal limits. One mesenteric lymph node was found to contain a focus of central necrosis containing intralesional bacteria and neutrophils surrounded by macrophages. Evidence of extramedullary hematopoiesis was noted in the spleens of one walrus and two bearded seals. In addition, the splenic artery of one of the same spleens had evidence of intimal thickening.

Mild trematode, cestode and nematode infections were identified in the duodenum of two bearded seals. Neutrophils were present in the crypts along with separation of the crypts by lymphoplasmacytic infiltration. A fluke was identified in the gall bladder of one of these animals. Both animals had mild lymphoplasmacytic infiltration of the pancreatic ducts. A consistent finding among all sections of liver examined from seals and walruses was focal areas

of hypertrophied Kupffer cells, occasional neutrophils and lymphoplasmacytic infiltration suggestive of parasitic migration through the liver parenchyma. Mild bile inspissation in the bile canaliculi was a common finding.

Mild dystrophic calcification was commonly identified in the renal pelvis of both seal and walrus kidneys. A single focus of lymphoplasmatcytic infiltration was identified in one seal and one walrus kidney. It was noted that Bowman's capsule in the walrus kidney was thicker than the same structure in the kidney of the bearded seal. This phenomenon was observed in all of the walrus kidneys sections studied and appears to be a normal finding.

A limited number of endocrine organs including thyroid and adrenal glands, and reproductive tracts were collected. Thyroid glands from three bearded seals were examined- nodular hyperplasia was observed in one set of glands, otherwise the thyroid glands were normal. Mild cortical hyperplasia was identified in the one adrenal gland that was examined histologically. Reproductive structures examined were normal.

Discussion

Postmortem findings in this study and morphological diagnosis are likely the result of normal social behaviors, bite wounds are especially common in the face and pelvic flippers. Biting is especially common in males during the breeding season. Although the histological findings in the seal suffering from pyogranulomatous panniculitis would suggest a penetrating wound consistent with vaccination, darting or tagging, no such activities have taken place in the group of seals examined. Seal tagging does not involve the use of tranquilizing agents. Furthermore tags are glued to the animals, not injected. Blubber biopsies are commonly taken during live capture and tagging events, however the multifocal nature of pyogranulomatous blubber lesions is not consistent with this practice. These lesions appear to be a common finding. During the summer of 2008, the researcher received the carcass of an adult male bearded seal judged to be unfit for consumption by the hunter. There was severe swelling of the right pelvic flipper, severe blubber serous atrophy, multifocal pyogranulomatous lesions in the dorsal and plantar flipper musculature and suspected osteolysis of the first digit (P1) distal phalanx.

Seals and walruses spend large amounts of time foraging at sea, however they are highly dependent upon ice and land substrates for reproduction, molting and resting (Wells et al, 1999). Marine mammal behaviors are changing in response to selection pressures exerted by a changing Arctic marine environment. In the fall of 2007, large scale walrus haulouts were noted for the first time ever on the Alaskan shores of the Chukchi Sea from Peard Bay to Cape Lisburne in response to pack ice dissipation and the disappearance of the last marginal and sparse ice (Fishbach et al, 2009). On September 14, 2009, USGS scientists discovered fresh carcasses of young walruses dispersed along the sandy beaches at Wainwright extending south to Icy Cape. The scientists documented the presence of 131 walrus carcasses. Most of the carcasses were from young animals based on aerial photodocumentation and examination of 12 of the carcasses. The exact cause of death has not been determined but it is speculated that trampling, exhaustion, or separation of calves from their mothers in response to environmental pressures including the loss of critical sea ice may have contributed to the largest walrus mortality event recorded on Alaskan shores of the Chukchi Sea (Fishbach et al, 2009). Rhabdomyolysis was a consistent histological finding in the skeletal muscle sections examined from the Wainwright bearded seal and walruses. There was also histological evidence of myocyte healing, likely occurring during time spent on land. Receding pack ice and loss of ice floes are forcing these animals to swim for greater distances to find suitable substrates critical for mating, birthing, molting and resting. It will be important to carefully observe skeletal muscle tissues from subsequent marine mammal hunting seasons for histological evidence of increased exertional rhabdomyolysis.

Antibodies to *Leptospira interrogans* serovar *grippotyphosa* have been demonstrated in a male subsistence harvested bearded seal and three walruses taken in the Northern Bering Sea near the coastal village of Gambell (63.781˚N, 171.736˚W) on St. Laurence Island (Calle et al, 2008; Calle et al, 2002). The prevalence of antibodies to *Leptospira* in Arctic seal and walrus populations is not well-documented because of difficulty obtaining fresh blood from these animals. Furthermore, the kidneys are an important delicacy in the Iñupiat diet. North Slope subsistence hunters have been extremely generous to the researcher, submitting sufficient quantities of renal tissues for examination. Infection with *Leptospires*, a potentially

zoonotic pathogen could have drastic consequences for the marine mammal population and the subsistence hunters and scientists who work with these animals. Leptospirosis has been reported in people that work closely with marine mammals, unfortunately many in the profession complain that medical professionals are not well-informed about potential marine mammal zoonotic pathogens (Hunt et al, 2008). The researcher did not find any renal lesions suggestive of *Leptospira* infection, however monitoring for the presence of the pathogen will continue in future marine mammal tissue collections. If the researcher does find renal lesions that suggest an infection, immunohistochemistry will be done on formalin-fixed renal tissues to demonstrate the possible presence of the microorganism.

Arctic indigenous coast people are aware of the risks for parasitic infection associated with consumption of subsistence resources. The consumption of *Trichinella spiralis*-contaminated walrus meat sickened people in Barrow in 1975 and again in 1976 (Margolis et al, 1979). Walrus and bearded seals commonly feed on benthic invertebrates in areas over the continental shelf, generally diving to depths of 100 meters or less (Wells et al, 1999). Scientists fear that the northwestward receding of pack ice towards deeper waters beyond the boundaries of the continental shelf will force walruses and bearded seals to change their feeding habits. Walruses are becoming increasingly predatory in their feeding habits, eating more ringed seals (Rausch et al, 2007; Wells et al, 1999). Dietary changes and predation may alter the resident helminthic fauna of the walrus and bearded seal gastrointestinal tract, increasing the likelihood that Alaska's native coastal populations will be at greater risk for infection with *Trichinella spp*. (Rausch et al, 2007). The expanding human and domestic animal presence in the Arctic and increased industrial activity may pollute the Arctic waters with novel parasitic pathogens. Antibodies to *Toxoplasmosis gondii* have been identified in bearded, ringed, and spotted seals and walruses from the coastal waters of Southeast Alaska to the Bering Strait from 1976-1998. *Neosporum caninum* antibodies have also been identified in three of the walruses from this same group (Dubey et al, 2003). Infection with *T. gondii* is most perplexing for a number of reasons. Domestic cats are the only hosts capable of shedding oocysts that can remain viable in the environment for great lengths of time, generally they only shed for 1-2 weeks (Bowman, 2009). The idea of land-based run-off of cat excrement

containing millions of oocysts in the Arctic waters to be concentrated by benthic organisms is hard to grasp. *N. caninum infection* in walruses is equally challenging to explain, given that the definitive host dog sheds much smaller quantities of oocysts (Bowman, 2009). In areas such as the North Slope, the potential role of the Arctic Fox in shedding of *N. caninum* needs to be addressed. *Sarcocystis* infection was first described in the salvaged carcass of an adult male bearded seal that had been killed by polar bears. The seal was found on Chukchi Sea ice (71˚26'N, 164˚52'-55'W) on July 21, 1977. A limited number of sarcocysts were found in the tongue skeletal muscle (Bishop, 1979). One tissue cyst resembling *T. gondii* was identified in the skeletal muscle of one of the Wainwright bearded seals while sarcocysts were consistently identified in the Wainwright bearded seal skeletal muscle tissues. Immunohistochemistry and PCR techniques will be used to determine if these sarcocysts are indeed different from other species reported in other marine mammals. Furthermore, little is known about Apicomplexan transmission and life cycles in marine mammals, but given their identification in these important subsistence resources, developing a greater understanding of their life histories is imperative.

The seals and walruses sampled were in good overall health and excellent body condition. Although the sample size is small, the tissues were of the highest quality because of the close relationship that scientists at the North Slope Borough Wildlife Department have worked to establish and maintain with the subsistence hunters. This relationship is allowing scientists to understand the complex anatomical adaptations that permit marine mammals to thrive in the marine environment. Furthermore, the researcher is developing an appreciation for morphological features at the microscopic level that are normal. This baseline information will allow scientists to recognize the effects of diminishing sea ice, increased water temperatures, ship traffic, and industrial development on the health of subsistence resources.

References

Bowman, D.D. (2009). Protozoans. *In* Georgis' Parasitology for veterinarians. 9th Edition. Saunders Elsevier, St. Louis, Missouri, pp 84-114.

Bishop, L. (1979). Parasite-related lesions in a bearded seal, *Erignathus barbatus*. Journal of Wildlife Diseases 15: 285-293.

Calle, P.P., Seagars, D.J., McClave, C., Senne, D., House, C., and J.A. House. (2008). Viral and bearded serology of six free-ranging bearded seals(*Erignathus barbatus)*. Diseases of Aquatic Organisms (81): 77-80.

Calle, P.P., Seagars, D.J., McClave, C., Senne, D., House, C., and J.A. House. (2002). Viral and bacterial serology of free-ranging Pacific walrus. Journal of Wildlife Diseases (38), 93-100.

Dubey, J.P., Zarnke, R., Thomas, N.J., Wong, S.K., Van Bonn, W., Briggs, M., Davis, J.W., Ewing, R., Mense, M., Kwok, O.C.H., Romand, S., and P. Thulliez. (2003). *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, and *Sarcocystis canis*-like infections in marine mammals. Veterinary Pathology (116): 275-296.

Fischbach, A.S., Monson, D.H., and C.V. Jay. (2009). Enumeration of Pacific walrus carcasses on beaches of the Chukchi Sea in Alaska following a mortality event, September 2009. U.S. Geological Survey Open File Report 2009-1291, 1-18.

Hunt, T.D., Ziccardi, M.H., Gulland, F.M.D., Yochem, P.K., Hird, D.W., Rowles, T., and J.A.K. Mazet. (2008). Health risks for marine mammal workers. Diseases of Aquatic Organisms (81): 81-92.

Margolis, H.S., Middaugh, J.P., and R.D. Burgess. (1979). Arctic trichinosis: two Alaskan outbreaks from walrus meat. Journal of Infectious Disease (139): 102-105.

Rausch, R.L., George, J.C., and H.K. Brower. (2007). Effect of climate warming on the Pacific walrus, and potential modification of its helminth fauna. Journal of Parasitology 93: 1247-1251.

Wells, R.S., Boness, D.J., and G.B. Rathbun. (1999). Behavior. *In* Biology of Marine Mammals. J. Reynolds and S. Rommel (eds.) Smithsonian Institution Press, Washington, D.C., pp 324-411.