



Beaufort Beluga Whales Health Assessment



2015-2016 FINAL REPORT

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 UNIVERSITY OF
SASKATCHEWAN

Health Assessment of the Beaufort Sea Beluga Whale in the Inuvialuit Settlement Region (Hendrickson Island)

2015-2016

Final report

Presented to:

Lisa Loseto, PhD
Central and Arctic Region
Freshwater Institute
Fisheries and Oceans Canada / Canada
501 University Crescent, Winnipeg
MB R3T 2N6, Canada.
Tel: 208 983 5135

Prepared by:

Émilie L. Couture, DVM, IPSAV, DES, Dip. ACZM
Stéphane Lair, DMV, DES, DVSc, Dip. ACZM
Canadian Wildlife Health Centre – Quebec
Faculté de médecine vétérinaire
Université de Montréal
3200 Rue Sicotte
Saint-Hyacinthe, Québec
J2S 7C6
Email: stephane.lair@umontreal.ca

In collaboration with:

Rozenn Le Net, MV, IPSAV, MSc.
Canadian Wildlife Health Centre – Quebec

Emily Jenkins, PhD, DVM, BSc
Associate Professor
Western College of Veterinary Medicine
University of Saskatchewan,
Saskatoon, SK

Adrián Hernández-Ortiz, DVM, MSc.
PhD Student
Western College of Veterinary Medicine

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EXECUTIVE SUMMARY

Being at the top of the food chain, beluga whales are important indicators of the quality (or health) of an ecosystem. Beluga whales are also a significant species for Inuvialuit communities, both culturally and as a food resource. Regional and global changes in the Arctic ecosystem can potentially affect the health of these valued animals. The purpose of this project was to perform a health assessment of beluga whales harvested as part of subsistence hunting by the local communities. This health assessment provides a *snap shot* of the health of the beluga whales harvested and investigated potential biological risks associated with the consumption of *mipku* and *muktuk*. The results of this health assessment, which was realized over two summers, could also be used as *base line* data to monitor changes in the health status of this population that will likely be affected by a changing environment over the years to come. In addition, data provided by whale monitors annually are of great interest, in addition to any anomaly of unknown origin that could be photographed, sampled and submitted for pathological analysis.

Key findings:

- Full necropsies were performed in a total of 26 Eastern Beaufort Sea (EBS) belugas harvested in 2015 and 2016.
- The whales examined during these seasons appeared to be healthy. With the exception of two animals, the beluga whales examined seemed to be in fair to excellent body condition.
- Based on a limited number (n=10) of weighed animals, scaled mass indexes were calculated and compared with alternative morphometric indexes. The “½ axillary girth/length” appears to be the best alternate morphometric index. It is recommended to weigh more animals to better evaluate the correlation between these alternative indices and the scaled mass index.
- Most of the pathological findings detected were associated with infections by different species of parasites. The parasitic burden was low and therefore not considered to have a significant impact on the general health and fitness of the whales. This suggests that a "healthy" equilibrium between the beluga whales from this population and their parasites.
- Parasitic conditions present included gastric nematodiasis (n=8), muscular sarcocystosis (n=6), verminous pneumonia (confirmed in n=20, but likely present in all individuals), renal nematodiasis (n=6) and lastly granulomatous lymphadenitis associated with trematodes (n=9), which has not been described in beluga whales. None of these parasites are known to transmit to people.
- Changes observed in the endocrine system are likely incidental, age-related findings and have been described in other beluga populations or cetacean species.
- Other observations generally pertained to normal physiologic processes or were age-related, such as multiple circular depressions of the skin surface, suggested to be associated with a normal molting process and reproductive senescence (menopause) in an older female. The other three sampled females were pregnant, and in one, ovarian cysts were noted.
- Samples from beluga harvested at two sites (Hendrickson Island and East Whitefish) were tested for food-borne parasites, the muscle-dwelling roundworm *Trichinella* and protozoan *Toxoplasma gondii*. From 33 serum samples, antibodies to *T. gondii* were detected in only one beluga (AREW15-06). From 31 heart samples and 23 brain samples, DNA of *T. gondii* was detected in only one heart sample (ARHI-DL-2015-06). *Trichinella* was not detected in tongue and diaphragm muscles examined from belugas harvested in 2015-2016.

PROJECT DESCRIPTION

Being at the top of the food chain, beluga whales are important indicators of the quality (or health) of an ecosystem. Beluga whales are also a significant species for Inuvialuit communities, both culturally and as a food resource. Regional and global changes in the Arctic ecosystem can potentially affect the health of these valued animals. The purpose of this project was to perform a health assessment of beluga whales harvested as part of subsistence hunting by the local communities. This health assessment provides a *snap shot* of the health of the beluga whales harvested and investigated potential biological risks associated with the consumption of *mipku* and *muktuk*. The results of this health assessment, which was realized over two summers, could also be used as *base line* data to monitor changes in the health status of this population that will likely be affected by a changing environment over the years to come.

Although the Eastern Beaufort Sea (EBS) beluga population appears to be sustainable, several other beluga whale populations across the Arctic are doing poorly. The southernmost population of beluga whales in the highly degraded St. Lawrence Estuary (SLE) ecosystem, province of Quebec, is also declining. Comparing the beluga whale populations of the Inuvialuit Settlement Region (ISR) and of the SLE could help to understand what the issues are with the endangered SLE population.

This program is the result of collaborations between Fisheries and Oceans Canada (DFO), the Hunters and Trappers Committee (HTC), the Fisheries Joint Management Committee (FJMC), the University of Saskatchewan, and the Canadian Wildlife Health Centre – Quebec Region (CWHC) located at the *Faculté de médecine vétérinaire* of the *Université de Montréal*.

MATERIAL AND METHODS

Beluga whales were harvested by local hunters in the water surrounding Hendrickson Island where they were hauled. After obtaining oral consent from the hunters, a full post-mortem examination was performed by CWHC (1-2) and DFO (2-4) staff. Whale monitors (2), as well as community youths (1-2) and an elder (1) were also fully involved in the process. To prevent contamination of food items, most of the examination and morphometric measurements were done after the collection of the meat and *muktuk*.

When possible, photographs of the carcasses (lateral, anterior and posterior views) were taken to enable a subjective assessment of the body condition. This assessment was done subjectively according to the appearance of the dorsal and postnuchal regions. Animals were either classified as in poor body condition (marked concavity of the dorsal area and depression of the postnuchal region), fair body condition (mild concavity of the dorsal area and depression of the postnuchal region), good body condition (no obvious concavity of the dorsal area and no depression of the postnuchal region) or excellent body condition (very rounded animal).

With the hunters' consent, some of the whales were weighed upon the landing of the carcass on the beach over the two sampling seasons. In 2015, the whale was secured in a large net and weight using an industrial scale (MSI-7300 Dyna-Link 2 Digital Tension Dynamometers) mounted on a rescue tripod (Skedco Sked-Evac Tripod) equipped with a lever hoist. The handling of the hoist necessitated the use of a bench. In 2016, the system was slightly modified; the tripod's legs were equipped with wood skids to prevent sinking of the legs in the sand, the lever hoist was replaced by a chain hoist and the net was replaced by a custom-built stretcher made of strong fabric and metal poles. In addition to the weight, several morphometric measurements were taken (Appendix I). One of the objectives of weighing the carcass was to be able to calculate a scaled mass index for each animal. This scaled mass index is calculated from the full mass and length and is proposed as the gold standard measure for the body condition in animals (Peig and Green 2009). The scaled mass index \hat{M}_i is derived from the application of a cross-multiplication to the allometric equation. It is calculated with the following formula: $\hat{M}_i = M_i * \left(\frac{L_0}{L_i}\right)^{b_{SMA}}$, where M_i is the mass of an individual, L_i is its length, L_0 is an arbitrary fixed length and b_{SMA} is the slope coefficient estimated from an SMA regression (Peig and Green 2009). In other terms, \hat{M}_i is a projection of the masses of individuals of different sizes on a single axis following the allometric curve. Practically, this removes

the effect of the structure on the masses, which can then be compared, as if all individuals measured the same length. To calculate this index a slope coefficient specific to the population need to be established. The number of animals that were weighted during 2015 and 2016 was too low to enable the determination of a slope coefficient specific to this population. Consequently, the slope coefficient specific for adult SLE beluga whales was used instead (slope coefficients of 3.3 and fixed length of 382.6 cm). Correlations between the scaled mass index and the following morphometric measurements were calculated using non-parametric Spearman correlation coefficient: "Epaxial muscle weight / length", " $\frac{1}{2}$ axillary girth / length", " $\frac{1}{2}$ maximal girth / length", "blubber depth axillary dorsal / length", "blubber depth axillary ventral / length", "blubber depth axillary mid-height / length" and "blubber depth dorsosacral / length". Correlations with " $\frac{1}{2}$ sacral girth / length" and "maximum width / length" were not done due to the limited sample size.

A full external and internal macroscopic examination was performed. The brain case was opened in all whales with a small axe. Ribs were either cut with a large garden secateurs (2015) or disarticulated with a knife (2016). During this examination, the presence of external and internal anomalies was documented. Photographs of these anomalies were taken when appropriate. Samples from the main organs were fixed in 10% buffered formalin for histopathological analysis. Eighteen sections of lungs representative of the entire parenchyma were taken following SLE beluga whales necropsy protocol. For each section, the percentage of surface area affected by lesions of pneumonia was estimated.

Selected parasites were fixed in alcohol for future identification. For food borne parasite testing (*Toxoplasma gondii* and *Trichinella* spp.), the Jenkins lab at the Western College of Veterinary Medicine tested samples from belugas harvested at Hendrickson Island (13 in 2015, 13 in 2016) and East Whitefish (5 in 2015, 3 in 2016) (total of 34 whales). Samples from the tongue and the diaphragm (20 g of each) were frozen, then tested for larvae of *Trichinella* sp. using the double separatory funnel digestion technique, considered the gold standard test. Brain and heart (100 g of each) were frozen for subsequent *Toxoplasma* sp. magnetic capture DNA extraction and qPCR testing. Whole blood was collected and centrifuged for subsequent serum testing for exposure (antibodies) to *Toxoplasma gondii* and thyroid hormones. The techniques performed to detect antibodies for *T. gondii* were enzyme-linked immunosorbent assay (ELISA), modified agglutination test (MAT) and immunofluorescence antibody test (IFAT). The complete necropsy protocols and field samples checklist used for both sampling seasons, as well as details on food-borne parasite testing, are presented in Appendices II to VII.

Fixed samples were prepared for histopathological analysis using a standard procedure. Special stains were used when warranted. Microscopic lesions are described in each individual's necropsy report along with macroscopic findings (Appendix VI). For the histological evaluation of the lung, the percentage of each section examined affected by pathological changes was calculated for each animal.



Weighing of a beluga whale in 2015

RESULTS AND DISCUSSION

SAMPLED BELUGA WHALES

In 2015, a total of 21 harvested whales were hauled on Hendrickson Island between July 9th and July 26th. A complete post-mortem examination was performed on 16 of these whales. All hunters agreed to have their beluga whales examined. Selection of the carcasses to be sampled was made based on the availability of human resources when each carcass was landed. In 2016, a total of 10 harvested whales were sampled between July 8th and 14th. Therefore, this report is based on the examination of a total of 26 carcasses done over two summers. Most sampled beluga whales were males (22/26) and all were mature adults (26/26). All the carcasses were freshly harvested, and therefore classified as code 2 (carcass in good condition / edible) on the standard carcass preservation scale (Geraci 2005).

SAMPLING AND EXAMINATION PROCESS

WEIGHT AND OTHER MORPHOMETRIC MEASUREMENTS

A total of 10 whales were weighed, 8 in 2015 and 2 in 2016. Weights ranged from 564 to 1147 kg. Total carcass length ranged from 342 to 446 cm, with 15/26 whales measuring over 400 cm. Unilateral epaxial muscle weight for the L1-L12 vertebral segment was obtained in 19 animals and ranged from 10 to 35 kg. All morphological measurements taken can be found in Appendix I.

As described above, the setup used to weight the whales evolved over time. Even if the efficiency of the process improved in 2016, the time needed to weight the beluga whales was still significant and represents a limitation for hunters. The setups used to weight the whales are presented in Figures 1 and 2. Here are some recommendations regarding the technique used to weight beluga whale carcasses in the field that contribute to increase the speed and safety of the process: The attachment of the weighing gear at the top of the tripod needs to incorporate all three hooks to distribute weight evenly. The chain hoist was more efficient than the lever hoist and allowed to stay further away from the tripod. The chain should be stored in a bucket to prevent dragging during transportation and to limit contamination of the mechanism with sand. The tripod is quite bulky and needs to be handled by three people. The bottom of the legs should be attached together with a chain to prevent them from spreading when the carcass is lifted. The skids of wood added under the feet of the legs helped prevent the sinking of the tripod legs into the sand as the carcass was lifted. The length of the stretcher, which was based on 2015 whales, was too short for large males in 2016. Depending on the water depth surrounding the whale, the clearance between the surface of the water and the maximal height of the tripod could be insufficient to fully raise the carcass above the water line. The use of a narrower stretcher (width = half of the girth of the largest whale) would decrease the clearance needed to properly weight the whale. The poles used to provide some rigidity to the stretcher were heavy, bulky to carry, and difficult to maneuver under water. A stretcher that would incorporate lighter poles would definitively help with this manipulation. Multiple trials may be needed at first to gage how to properly distribute the weight of the whale regarding the attachments to allow for the more horizontal lift possible. Although weighing whales in a field setting remains a challenge and requires some patience, it is possible with experience, proper equipment and technique.

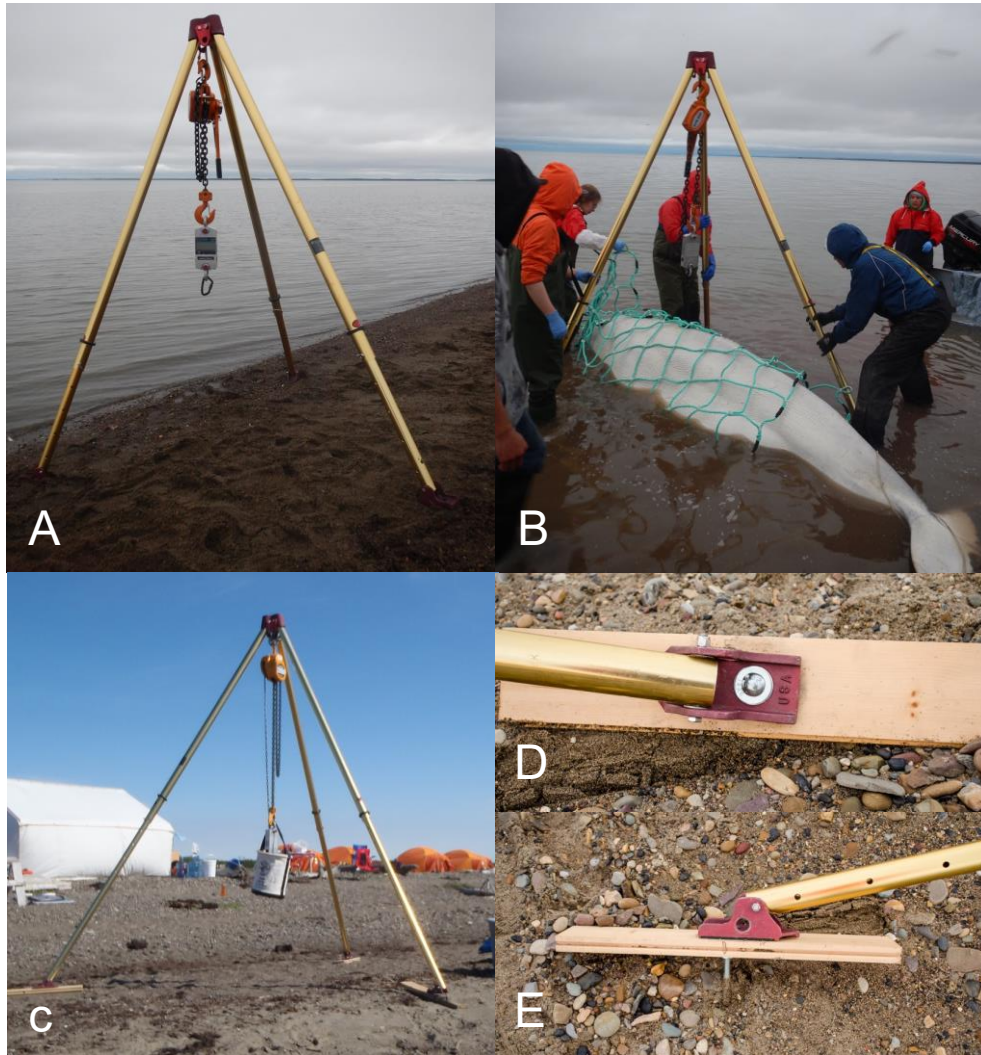


Figure 1. Settings used to weight the beluga in 2015 (A, B) and 2016 (C), showing tripod, scale and lever hoist (2015) or chain hoist (2016). The chain is housed in a basket when not in use to prevent sand from soiling the mechanism. Details of the wood skids attached under the legs to prevent the sinking into the sand (D, E).

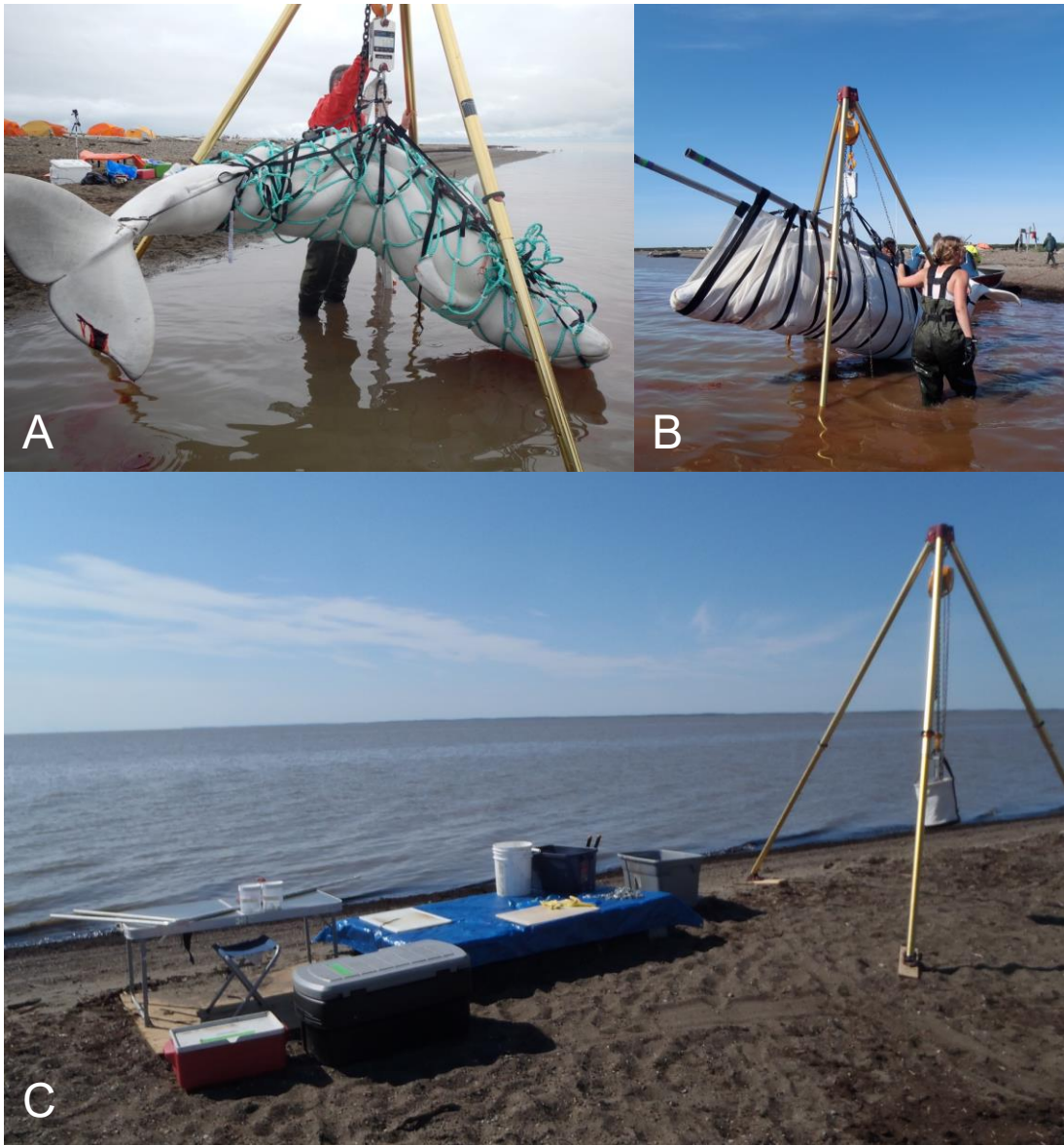


Figure 2. Weighing of a beluga in 2015 (A) and 2016 (B). Subsampling setup in 2016 (C).

GROSS NECROPSY AND SUBSAMPLING

Each organ was removed from the body cavities to be examined. To facilitate the management of these samples and prevent contamination with sand, organs were placed in large plastic bins in 2016 (purchased at North Mart, Inuvik). These bins were large enough to contain most viscera from a single individual. They were helpful in keeping all organs together and away from the tide until they could be examined and subsampled. This process was especially efficient during multiple landings in a short period. Following sub-sampling of organs, the leftover tissues were kept in the bins to facilitate their disposal away from the camp.

Subsampling of organs on cutting boards on an elevated plywood surface covered with a plastic tarp limited sample contamination with sand in 2016. Subsampling was generally performed by two people, while a third 'clean hands' was in charge of photographs, labelling and recording of relevant information (subsampling setting, Figure 2).

In several cases, problems with serum/plasma extraction were reported following centrifugation due to a failure of "separation" of the blood. To prevent this, it is recommended to fill blood tubes with anticoagulants (e.g. green-topped – Heparin) only up to the recommended level to ensure a proper blood: anticoagulant ratio. The collection of blood with a catheter tip syringe followed by the filling of the wanted number of tubes allows a better control of the amount of blood poured as opposed to sampling the blood directly from the carcass with the tubes. Overfilling of all kind of tubes may also result in difficulty closing the tubes and increased risk of the cap popping off during processing. It is also recommended to wait at least 30 minutes before spinning the red-topped blood tubes to allow the formation of a clot (these blood tubes should be left at 'room temperature' and not chilled, and should therefore likely be kept warm due to generally cool weather on the island). It should be mentioned that cetaceans lack clotting factor XII, which has been reported to be associated with difficulties in extracting serum. To alleviate this, it is recommended to collect whole blood in an orange-topped tube, which has added thrombin to induce clotting and facilitate serum separation (St Leger 2018). Hemolysis of the blood could also be associated with an apparent lack of separation between the blood cells and the serum. Due to the high concentration of heme in the blood cells of cetacean, hemolysis could be associated with a very dark staining of the serum/plasma giving the impression that the sample has not separated. Hemolysis may interfere with the results of some assay (spectrophotometric). In addition, hemolysis can cause a false increase in plasmatic / serum of analytes that are present in large concentrations within erythrocytes, such as potassium (Weiser 2012). Mixing blood with water can potentially cause the osmotic lysis of the erythrocytes, and therefore hemolysis. Consequently, contamination of blood sampled with water should be avoided, by sampling directly in large intact vessels with a catheter tip 30 ml syringe (be careful to avoid areas damaged by gunshots that might have allowed sea water in the body cavity).

HEALTH ASSESSMENT AND PATHOLOGIC FINDINGS

MORPHOLOGIC MEASUREMENTS AND BODY CONDITION

A subjective body condition was attributed to 24 of the whales. Seventeen animals were considered to be in excellent or good body condition, whereas five animals were evaluated to be in fair body condition and two animals were scored as in poor body condition (Appendix I). A scaled mass index was determined in eight animals (Appendix I). The median scaled mass index of beluga whales scored to be in excellent or good body condition (670 kg) was higher than the median scaled mass index of beluga whales evaluated to be in fair or poor body condition (639 kg). However, if we assumed that the scaled mass index is the gold standard for the assessment of nutritional status in this species, the relatively wide overlap observed between the two groups (excellent/good VS fair/poor) for this parameter suggests that the subjective evaluation of the body condition has some limitations (Figure 3). Nevertheless, as demonstrated for carcasses of SLE beluga whales (Larrat 2019), animal in poor body condition are correctly classified by subjective evaluation based on external morphometric characteristics. Consequently, since only two of the 24 carcasses evaluated was estimated to be in poor body

condition, the overall nutritional status of this population in 2015 and 2016 was evaluated to be adequate (assuming that the sampled beluga whales are representative of the actual population).

The scaled mass indexes calculated for the Beaufort beluga whales were compared to the scaled mass indexes of the SLE beluga whales that died of known causes (Figure 4). These SLE beluga whales were separated in two groups: animals that died following an illness of short duration or acute (therefore likely to be in adequate body condition) and animals that died following long lasting or chronic illnesses (therefore likely to be thin or emaciated). Interestingly, the range of the scaled mass indexes of the harvested Beaufort beluga whales is similar to the range of the SLE beluga whales that died of chronic diseases (and therefore likely thin). Also, when we compare the weights of animals in these two populations we see that, for equivalent length, harvested Beaufort beluga whales have weights similar to SLE beluga whales that died following chronic illnesses (Figure 5). Since most of the harvested beluga whales from the Beaufort population were estimated to be in good body condition, these observations essentially indicate that the normal morphology of Beaufort beluga whales differs from the SLE population; Beaufort beluga whales are slenderer than SLE beluga whales. This morphometric difference was also observed when comparing the different girth measurements of the Beaufort beluga whales (smaller circumferences) to the SLE beluga whales. This difference might be associated with the migratory nature of the Beaufort population; migrating beluga whales travel long distances, which favor slender animals with higher hydrodynamic coefficient.

Figure 3. Boxplots presenting the distribution of the scaled mass indexes of beluga whales subjectively evaluated in excellent/good or fair/poor body condition. Horizontal lines represent the medians, box limits indicate lower and upper quartiles (25% and 75%), whiskers indicate the range of the distribution.

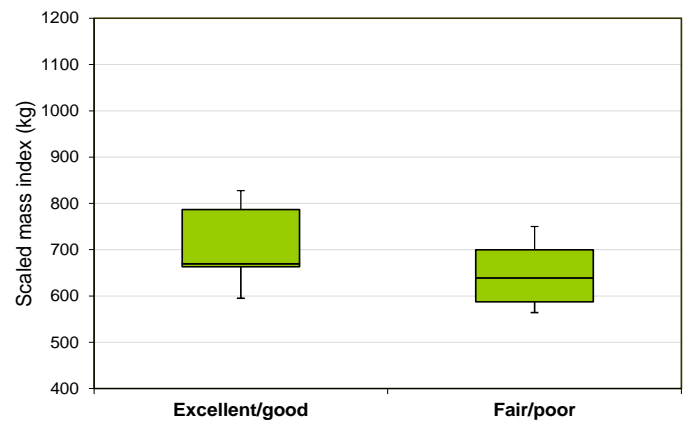


Figure 4. Comparison of the scaled mass indexes of beluga whales harvested from the Beaufort population (HI – harvested) and the SLE beluga whales that died acutely of following a chronic disease. Horizontal lines represent the medians, box limits indicate lower and upper quartiles (25% and 75%), whiskers indicate the range of the distribution.

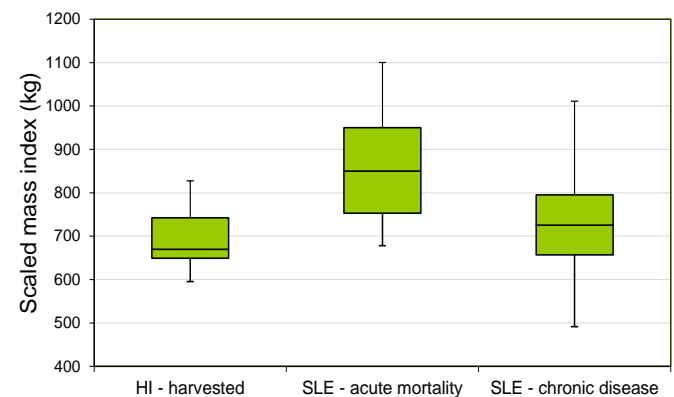
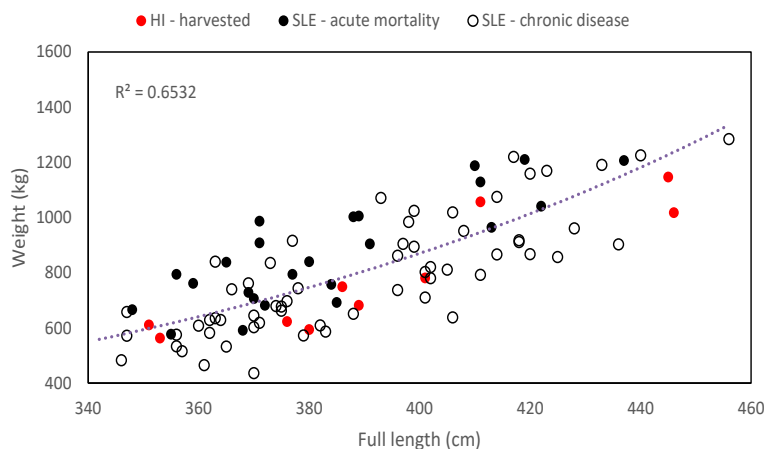


Figure 5. Weight and length of beluga whales harvested from the Beaufort population (HI – harvested) and the SLE beluga whales that died acutely or following a chronic disease.



One of the objectives for calculating scaled mass indexes was to be able to evaluate if alternate morphometric measurements could be used to objectively evaluate body condition in Beaufort beluga whales. The results of the correlation between the scaled mass index (the gold standard) and different morphometric indexes are presented in Table 1.

Table 1. Correlation between the scaled mass index and different alternative morphometric measurements.

Alternative morphometric indexes	Spearman correlation coefficient	P values
Epaxial muscle weight / length	0.11	0.82
½ axillary girth / length	0.75	0.05
½ maximal girth / length	0.33	0.42
Blubber depth axillary dorsal / length	0.29	0.5
Blubber depth axillary ventral / length	0.12	0.78
Blubber depth axillary mid-height / length	0.11	0.82
Blubber depth dorsosacral / length	0.57	0.14

Even if these correlations are based on a small sample size, they suggest that the "½ axillary girth / length" is the best alternate morphometric indexes that could be used as a proxy for the scaled mass indexes. This finding is in agreement with data from the SLE beluga population for which a significant coefficient correlation of 0.70 was found between the scaled mass index and the scaled axillary girth for beluga with a total length > 290 cm (Larrat 2014). In the SLE beluga whales a similar significant correlation (0.68) was obtained for the scaled sacral girth and for the scaled maximal girth (0.82). Due to the small sample size it was not possible to validate this index in the Beaufort beluga whales. Nevertheless, if we extrapolate findings from the SLE beluga whales, the "½ axillary girth / length" is likely to be a valid alternative estimation measurement of the body condition of animals. Interestingly, girth indices in this population were also considered to be a valuable body condition indicator in parallel studies, being more responsive than blubber thickness indices while also being a significant predictor for the fatty acid signatures (Choy 2017, MacMillan 2018). Although these measurements were obtained from the same population, the correspondence with the girth described in MacMillan ("measured behind

pectoral fins from mid-chest to the dorsal ridge”, axillary girth depicted on monitoring form) and Choy (“maximum half girth (indicated by the dorsal ridge)”) with either axillary girth or maximal girth presented here is uncertain.

Low correlations were observed between the scaled mass index and the "epaxial muscle weight / length" and the "axillary blubber depths / length" (ventral, dorsal and mid-height). This suggests that these morphological indexes are not sensitive indicators of the nutritional status of the animal. This observation is similar to what have been described in SLE beluga whales in which scaled axillary blubber depths do not correlate well with the scaled mass index (Larrat 2014). A higher, but not significant correlation was however observed with the "Blubber depth dorsosacral / length" suggesting that blubber dynamic varies from one anatomic location to the other.

Based on the observations made on the beluga whales from the SLE and the Beaufort populations it is recommended to continue to evaluate morphometric measurements on harvested animals from this population, including total weight, in order to better validate these alternate morphologic indices. In the mean time, "½ axillary girth / length" and "blubber depth dorsosacral / length" should be seen as the best alternate indices of relative body condition. Evaluation of the different morphologic indices over time might provide information regarding the variation in the nutritional status of animals in this population.

PATHOLOGIC FINDINGS

Overall, the animals examined appeared to be in very good health. Only a few grossly visible incidental abnormalities were detected. These anomalies were mainly associated with infection by different species of parasites, most of them already documented in beluga whales. Detailed information regarding the different findings and their significance for the health of the animals are listed below per body system. A summary of these findings is presented in Table 2.

INTEGUMENTARY SYSTEM

Multiple **circular depressions of the skin** surface were noted in most of the whales (Figure 6) and were often observed on the dorsal portion of the body. Histopathological examination of these cutaneous depressions was suggestive of a normal molting process. Similar circular depressions have been reported in Hudson Bay beluga whales in their summer estuarine passage, when a layer of degenerating cellular debris is often noted along the middorsal ridge. It is suggested that this might be associated with previous damage to the *stratum externum* (the outermost layer of three epidermal layers) from whales using their backs and heads to break through thin ice, causing a degeneration of the underlying *stratum intermedium* that eventually sloughs off once in the estuary. The temperature of the water also has an effect on the rate of cellular dissociation and germinal cell proliferation (St Aubin 1990).

One whale had an inflammatory reaction by the lip (**cheilitis**) associated with the presence of foreign bodies. A **nonspecific dermatitis** and superficial skin erosions, were observed in two additional cases. These lesions were not clinically significant for the animals.

Table 2. Diagnostics and findings of interest for beluga whales harvested off Hendrickson Island in the summer of 2015 and 2016.

ARHI-DL	Active lactation	Pregnancy	Skin circular lesions (molt)	Squid beaks in stomach	Lung indurations	Verminous pneumonia	Gastric nematodiasis	Muscle sarcocystosis	Kidney nematodiasis	Mesenteric lymph nodes nodules	Nematodiasis of the pterygoid sinuses	Pituitary cystic lesion	Adrenocortical hyperplastic/cystic lesions	Hyperplastic/cystic lesions of the thyroid	Other findings
2015-02	NA	NA	x					x				x	x		Splenic fibrosis (scar)
2015-03	NA	NA	x												Splenic hemangioma
2015-04	NA	NA			x	x	x		x			x	x		
2015-05	NA	NA				x									
2015-06	Full	x	x					x				x	x		Ovarian cysts
2015-08	NA	NA	x			x									Nodule within mesentery, kidney nodule
2015-12	NA	NA	x						x	x			x		
2015-13	NA	NA	x												Cheilitis with foreign body, liver scar
2015-14	Partial		x			x							x	x	
2015-15	Partial	x						x		x			x		
2015-16	NA	NA	x	x		x	x	x		x		x	x		
2015-17	NA	NA	x			x			x	x		x		x	
2015-19	NA	NA	x		x	x	x	x				x	x	x	Mild dermatitis
2015-20	NA	NA	x		x	x		x					x		Focal myositis
2015-21	NA	NA	x		x	x				x					
2015-22	NA	NA		x	x	x				x				x	Focal orchitis, cerebral senile changes
2016-02		x	x												
2016-03	NA	NA	x		x	x			x						Oesophageal ulcers- uncertain etiology
2016-04	NA	NA	x				x		x	x			x		
2016-05	NA	NA	x		x	x			x	x				x	Mild cholangitis
2016-06	NA	NA	x			x	x					x	x	x	
2016-07	NA	NA	x			x	x					x	x	x	
2016-08	NA	NA	x			x	x		x	x	x	x	x	x	Accessory spleen
2016-09	NA	NA	x			x	x		x				x		
2016-10	NA	NA	x			x						x		x	Focal granulomatous gastritis
2016-11	NA	NA	X		x	x							x	x	Cerebral senile changes

NA: not applicable



Figure 6. Circular depressions of the skin surface believed to be associated with normal molting processes. Dorsal and cranial disposition of lesions (A) and close-up appearance (B, C).

MALE REPRODUCTIVE SYSTEM

Twenty-two of the examined whales were males. No macroscopic lesion to the reproductive system was observed. In all cases for which a histologic section was examined, there was no sperm stored in the epididymis (21/21) and seminiferous tubule sperm production was minimal to nonexistent (22/22). In three cases, the testicular interstitial connective tissue appeared to be more abundant. An **epididymal epithelial cyst** (1/21) and **multifocal foci of mineralization** (6/21) were noted. A focal **lymphoplasmacytic orchitis** was also noted in one individual (1/22).

Mating in beluga whales generally occur in the spring, with geographically variable timing (Stewart 1989). In a captive beluga whales housed at a more southern latitude (California, USA), ejaculate volume, sperm concentration and total spermatozoa were higher from January to June (O'Brien 2008). The minimal spermatozoid production observed in the testis at the time of harvest (July) is in accordance with this. Increase in interstitial connective tissue (fibrosis) and mineralization are generally associated with age

(Foster 2016). Focal accumulations of lymphocytes in the testicular interstitium is usually an incidental finding in most species.

FEMALE REPRODUCTIVE SYSTEM

Four females were evaluated. An early **pregnancy** with a male foetus was confirmed in three of the four females. One foetus was measured (18.5 cm) and weighed (141 g), and the other two were of a similar estimated size (≈ 20 cm). Two pregnancies were in the right uterine horn whereas one was likely in the left horn, based on reported *corpus luteum* (CL) location. Pregnancies were associated with 1 (2/3) or 2 (1/3) CL on the corresponding ovary. Accessory CL are reported in beluga whales. They have been noted on 25% of sexually mature eastern Chukchi Sea beluga whales and one individual had 5 CL. Accessory CL result from the luteinisation of an accessory follicle in addition to the one associated with the pregnancy. The pregnancy rate cannot be determined adequately from our small sample size. It has been determined to be 0.41 in eastern Chukchi Sea beluga whales, which would be consistent with a calving interval of two to three years (Suydam 2009).

In the female with two CL, four cystic structures were noted in the contralateral ovary. **Ovarian cysts** have been reported in dolphin species and appeared to be associated with a reduced pregnancy rate in dusky dolphins (Sweeney 1975, Van Bresse 2000). The nonpregnant female, 55 years old, appeared to have quiescent ovaries: no follicle development was noted and multiple fibrous scars (*corpus albicans*, indicative of past pregnancies) were noted. This female was probably menopausal.

Various intensities of **lactation** were observed in three of the four females, including two pregnant and one not pregnant. Lactation was abundant in one of the pregnant cows and the mammary gland appeared partially active in another pregnant cow and in the presumed menopausal female. This observation suggests that they were nursing an older juvenile, as weaning appears variable and may occur between the 1st and 4th year of life (Matthews 2015). In addition, or alternatively, allonursing could be another reason for lactation in either of these individuals, as it has been observed in captivity (Leung, 2010). Findings associated with the female reproductive tract are detailed in Table 3.

Table 3. Details of findings relative to the female reproductive system.

	ARHI-DL-2015-06	ARHI-DL-2015-14	ARHI-DL-2015-15	ARHI-DL-2016-02
Age	45	55	35	ND
Pregnancy	Yes	No	Yes	Yes
Side	Left (likely)	NA	Right	Right
Foetus - sex	Male	NA	Male	Male
Foetus - size (cm)	20*	NA	20*	18.5
Foetus - weight (g)	ND	NA	ND	141
Ovary	Cyst (right), CL x2 (left)	CA - no follicle development	CL - (right likely)	CL - right
Mammary gland	Active	Partial activity	Partial activity	Inactive

*Estimation, ND: not determined, NA: not applicable CL: *corpus luteum*, CA: *corpus albicans*

ENDOCRINE SYSTEM

Thyroid glands: The thyroid gland weight was obtained in 14 animals. Median weight was 146 g (range: 64-323 g). Cystic or hyperplastic lesions were observed in 10 of the 26 thyroid glands examined. **Cystic follicles** (≥ 1 mm) were detected in 8 individuals, while **nodular hyperplastic lesions** were present in 5 animals. Examples of macroscopic changes in the thyroid can be found in Figure 7. **Interstitial fibrosis** and **discrete mineralization** were noted in one individual each. In addition, a subjective evaluation of the variation in follicular size was done and is detailed in Table 4. Moderate to marked variation in follicular size was associated with invaginations and marked activity of the follicular epithelium. Follicular cysts and adenomatous hyperplastic lesions have been described in both arctic (Hudson Bay) and SLE beluga whale populations. In these reports, the intensity of adenomatous hyperplastic changes was correlated with age (Mikaelian 2003). The clinical implication and etiology of these cystic and hyperplastic changes in the thyroid glands remain unclear.

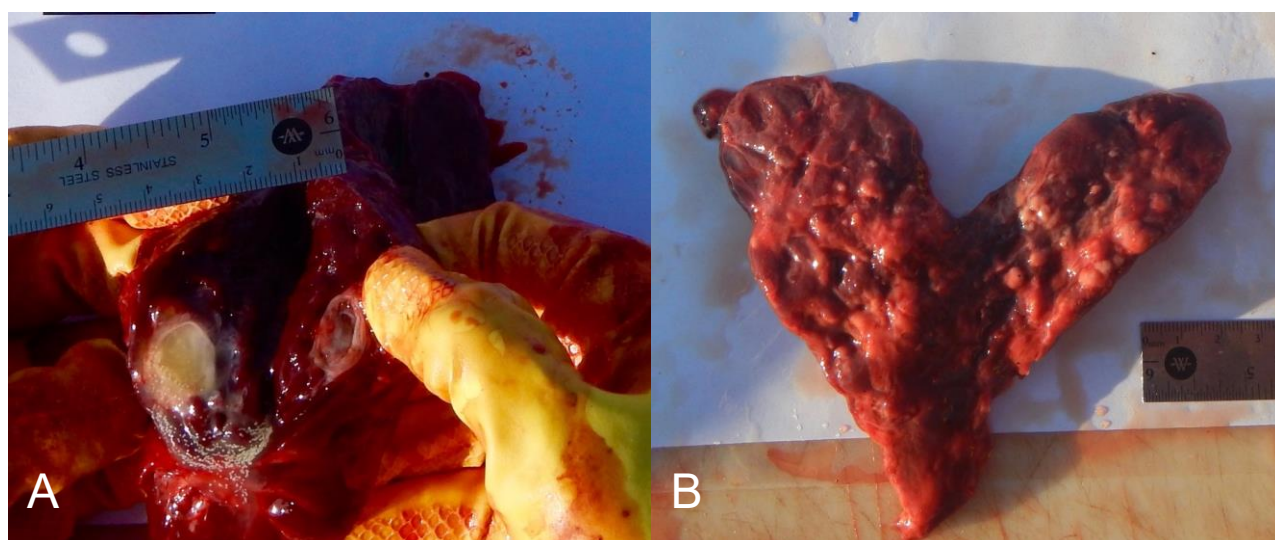


Figure 7. Macroscopic appearance of thyroid lesions in beluga whales. Thyroid cyst (A) and thyroid nodular hyperplasia (B).

Adrenal glands: The adrenal gland weight was obtained in all ten whales harvested in 2016. **Adrenocortical cysts** and **nodular cortical hyperplasia** were observed in 15 and 2 individuals respectively. Adrenocortical cysts and cortical hyperplasia have been described in SLE beluga whales (Lair 1997). Even if a link between these changes and the exposure to adrenocorticolytic contaminants has been proposed, this remains speculative. These changes, of unknown clinical significance, are actually probably part of a normal aging process.

Pituitary glands: **Pituitary gland cysts** filled colloid were detected in 9 of the 25 individuals in which the pituitary gland was examined. Psammoma bodies were noted in 5 individuals. These type of histological changes of unknown clinical significance have been described in other cetacean species (Cowan 2008).

While ages for the 2016 beluga whales are missing to complete the assessment, it is interesting to note that all but one whale with lesions detected on at least one type of endocrine gland in 2015 are over the age of 25. Of the 19 individuals with a lesion in at least one glandular tissue, the majority (12/19) had

concurrent lesions in at least another gland. Details of findings related to the endocrine system are presented in Table 4.

Table 4. Summary of endocrine system findings and morphological data.

#ARHI DL-	Age (years)	Thyroid gland weigh (g)	Left adrenal (g)*	Right adrenal (g)*	Thyroid follicle size variability	Thyroid hyperplasic/ cystic lesions	Pituitary cystic lesions	Adrenocortical hyperplasic/ cystic lesions
2015-02	25	ND	30,3	25,5	Normal to mild		x	x
2015-03	20	ND	27,0	21,2	Normal			
2015-04	32	ND	44,2	47,6	Normal		x	x
2015-05	17	ND	19,2	11,2	Mild			
2015-06	45	ND	29,5	37,5	Normal		x	x
2015-08	22	ND	22,3	28,1	Normal			
2015-12	20	ND	21,2	21,0	Normal			x
2015-13	22	ND	25,5	28,4	Normal			
2015-14	55	ND	29,9	27,8	Marked	x		x
2015-15	35	ND	21,8	18,6	Moderate			x
2015-16	39	86	32,8	31,4	Moderate		x	x
2015-17	25	149	ND	24,0	Mild	x	x	
2015-19	34	220	29,4	29,5	Mild	x	x	x
2015-20	27	116	33,9	37,6	Normal			x
2015-21	23	118	21,6	22,3	Normal			
2015-22	31	142	31,1	30,3	Mild	x		
2016-02	NA	64	16,5	17,9	Normal			
2016-03	NA	96	29,8	31,0	Normal			
2016-04	NA	151	26,6	30,4	Normal			x
2016-05	NA	187	35,2	41,1	Moderate to marked	x		
2016-06	NA	232	42,2	38,7	Moderate	x	x	x
2016-07	NA	195	30,4	35,4	Mild	x	x	x
2016-08	NA	ND	46,0	34,2	Moderate	x	x	x
2016-09	NA	323	27,8	29,2	Moderate to marked			x
2016-10	NA	ND	24,7	26,6	Normal	x	x	
2016-11	NA	93	44,7	53,8	Moderate	x		x

*Formalin-fixed tissue weight; NA: not available, ND: not determined.

DIGESTIVE SYSTEM

Oesophageal ulcers were observed in one individual. No obvious etiology could be determined from the microscopic examination of tissues, and unfortunately no frozen samples were available for further diagnostic.

The only food items detected in the stomach examined was a small quantity of cephalopod (squid) beaks in two individuals. This low percentage of animal with food contain is in agreement with what have been described for this location and dates (Harwood 2015). The very low quantity of stool concurrently found in the intestine is also suggestive that the animals have not significantly feed for a certain time period.

Gastric nematodiasis was observed in 8 animals. Nematodes of the *Anisakidae* family were found in small numbers, mostly in the first gastric compartment. Gastric nematodes described in beluga whales include *Contraecum* sp. which was the most prevalent and abundant helminth encountered in Beaufort Sea beluga whales (Wazura 1986). *Anisakis simplex* were the most prevalent and numerous parasites found in stranded beluga whales from the St. Lawrence estuary between 1986 and 1993 (Measures 1995). Nematodes from the *Anisakidae* family often cause low-grade ulcerative gastritis of the first compartment. Even if a few instances of perforated ulcerations of the stomach and intestines have been reported in St. Lawrence beluga whales, the vast majority of the infections are believed to have low clinical significance (Lair 2016). The parasitic burden observed in the Beaufort beluga whales harvested in 2015 and 2016 was relatively low, which suggests an equilibrium between these parasites and their host (in other words, beluga whales did not seem to be overly affected by these parasites). The absence of lesions associated with the presence of these parasites is also in agreement with this observation. Nevertheless, in a beluga sampled opportunistically in 2019, nematodes were associated with severe ulceration and thickening of the gastric wall (Figure 8), which suggests that infections by these parasites can be associated with a physiological cost to some of the animals.

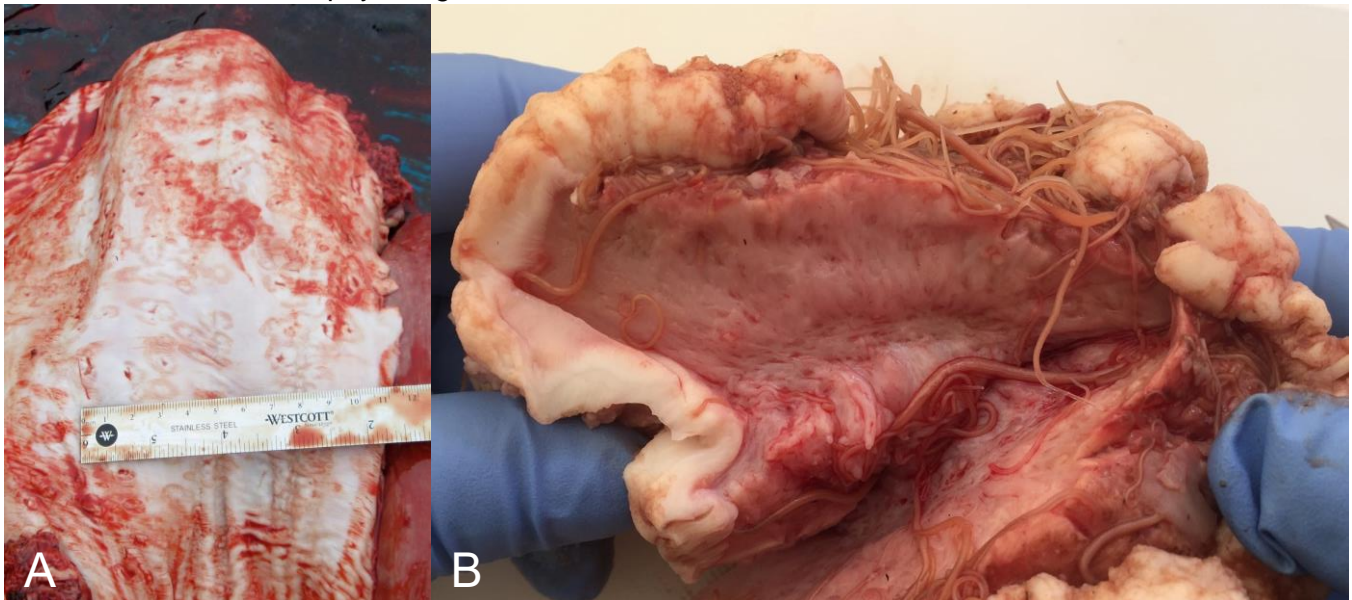


Figure 8. (A) Appearance of oesophageal ulcers of undetermined etiology. (B) Cut section of a chronic stomach ulcer due to the presence of nematodes (*Anisakidae*) in a whale harvested in 2019. Note the presence of the markedly thickened gastric wall underlying the ulcerated epithelium. Even if nematodes were frequently observed in animals harvested in 2015 and 216, gastric ulceration was not observed in any of the animals.

MUSCULOSKELETAL SYSTEM

Protozoan parasites of the genus *Sarcocystis* sp. were detected during the microscopic examination of the muscle from 6 of the beluga whales, all harvested in 2015. These protozoan cysts were not associated with gross lesions or inflammatory changes. *Sarcocystis* sp. is a protozoan parasite with an obligatory 2-hosts lifecycle that is found worldwide. There are over 150 known species of *Sarcocystis* sp. Intermediate hosts, which are usually herbivore mammals but may also include humans, birds, reptiles and potentially fish, are infected through consumption of food contaminated with sporocysts from infected definitive hosts' fecal material. During subsequent cycles of asexual development, the organism eventually reaches muscle (smooth, skeletal or cardiac) and sometimes neural tissues where it forms a sarcocyst that can persist from months to years (Fayer 2015). *Sarcocystis* sp. of uncharacterized species have been documented previously in beluga whales from the St. Lawrence population. The cycle of these *Sarcocystis* sp. has not been determined. Since the microscopic examination of sections of muscle tissue does not have a high detection sensitivity, the true prevalence of *Sarcocystis* sp. in Beaufort Sea beluga whales is most likely higher than what was documented. Sexual reproduction of this parasite occurs in the digestive tract of the definitive host following the consumption of infected meat. Polar bears, orcas, Greenland sharks, birds and crustaceans have been proposed as definitive hosts for beluga whale *Sarcocystis* sp. (De Guise 1993).

Only two species of *Sarcocystis* spp. found animals are known to be infect humans through the consumption of undercooked meat (*Sarcocystis hominis* from beef, and *Sarcocystis suihominis* from pork). Intestinal sarcocystosis in people could be associated with digestive signs, such as nausea, vomiting and enteritis. However, most of the infection in people will remain asymptomatic (Fayer 2015). There is no report of human infection from *Sarcocystis* sp. from marine mammals or from free-ranging games. Therefore, these parasites are unlikely to represent a health risk for harvesters.

RESPIRATORY SYSTEM

Various intensities of pneumonia were observed in all examined whales. The pulmonary inflammatory changes observed were similar in all animals. Nematodes (lungworms) or fragments of nematodes were associated with these lesions of pneumonia in 20 of the beluga whales. Consequently, it is believed that all the beluga whales examined were affected by some levels of **verminous pneumonia**. The morphologies of the parasites present are characteristic of nematodes from the genus *Stenurus* sp. and *Halocercus* sp. Grossly these parasitic lesions are characterized by localized indurations in the lung parenchyma (Figure 9). Worms (*Stenurus* sp.) can also occasionally be seen in the large airways (bronchi). Details on findings related with the histopathologic examination of the 18 lung slides per animals are presented in Table 5.

Halocercus sp. and *Stenurus* sp. are commonly found in the lung of stranded beluga whales from the SLE and Cook Inlet populations. In these two populations, verminous pneumonia is one of the most common cause of mortality, especially in juvenile animals in which the parasitic load can be significant (Burek-Huntington 2015, Lair 2016). The parasite burden and the extent of the lesions associated with these parasites were quite low in the beluga whales landed on Hendrickson Island in 2015 and 2016. Actually, the percentages of sections affected by the lesions of pneumonia vary from 0 to 70%. The median percentages per animal (for the 18 sections of lung) vary from 0 to 10%, with an overall median percentage of 4%. Consequently, even if all parasitic infections do come with an energy cost for the host, we believe that the intensity of the infections observed in these animals were too low to have an impact on the fitness of the individuals. This observation suggests that the host (the beluga) are well adapted to these parasites.

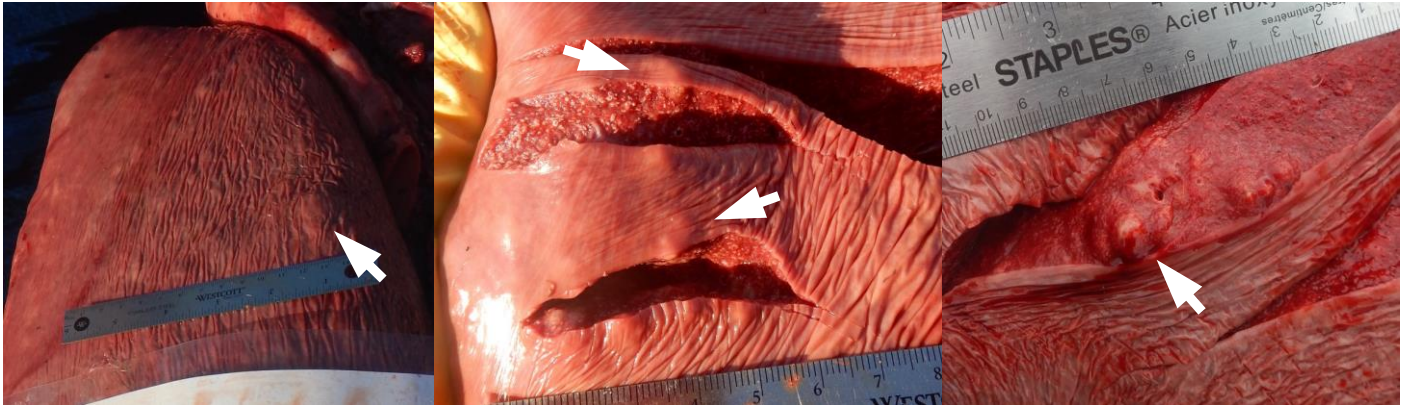


Figure 9. Nodules or indurations (arrows) associated with verminous pneumonia in beluga whales.



Team members getting ready to sample the whale while meat is being harvested by hunters.

Table 5. Summary of findings observed in the lungs of examined beluga whales. Eighteen sections of lungs were examined per animals.

#ARHI DL-	Percentage (%) of the surface area affected by lesions for each section of lung				Parasite present*	
	Min	Max	Median	Mode	<i>Stenurus</i> sp.	<i>Halocercus</i> sp.
2015-02	0	5	0	0		X
2015-03	0	15	5	1		
2015-04	0	5	1	0	X	X
2015-05	0	50	1	0	X	X
2015-06	0	20	5	5		
2015-08	0	25	5	5	X	X
2015-12	0	30	1	1		X
2015-13	0	15	1	1		
2015-14	0	25	5	0		X
2015-15	0	5	1	1		
2015-16	0	10	1	1	X	X
2015-17	0	20	3	1		X
2015-19	0	30	5	5	X	X
2015-20	0	15	1	1	X	X
2015-21	0	60	5	5	X	X
2015-22	1	70	10	5	X	X
2016-02	0	5	3	5		
2016-03	1	30	1	1	X	
2016-04	0	5	0	0		
2016-05	0	10	5	5	X	X
2016-06	5	40	6	5		X
2016-07	0	25	5	5	X	X
2016-08	0	20	5	5	X	X
2016-09	0	5	1	5		X
2016-10	0	50	5	5	X	X
2016-11	0	40	10	0	X	X
Overall / Total	0	70	4	5	14	19

*Presence of *Halocercus* sp. was either confirmed or suspected based on the presence of section of nematodes or of granuloma surrounding mineralized material (likely degenerate parasite).

URINARY SYSTEM

Renal nematodes of the genus *Crassicauda* were detected in 8 individuals. *Crassicauda* spp. are a spirurid nematodes found in mysticetes and odontocetes and are generally associated with the urogenital system and mammary glands, although it may also be found in the cranial sinuses. These nematodes cause white nodules in the renal parenchyma (Figure 10). Multifocal, small foci of mineralization were noted in the renal medullar parenchyma in 6/26 individuals and were considered incidental. *Crassicauda giliakiana* is the species usually associated to the urogenital system in beluga whales (Measures 2018). This parasite can cause significant lesions in beluga whales; it has been reported to be a contributing cause of death in 16 out of the 31 beluga whales stranded in Cook Inlet (Burek-Huntington 2015). In all the cases observed in 2015 and 2016, lesions associated with *C. giliakiana* were mild (< 10 nodules per kidney) and were considered to be incidental. Interestingly, this parasite seems to be specific to Arctic beluga whales since it has never been described in St. Lawrence beluga whales.

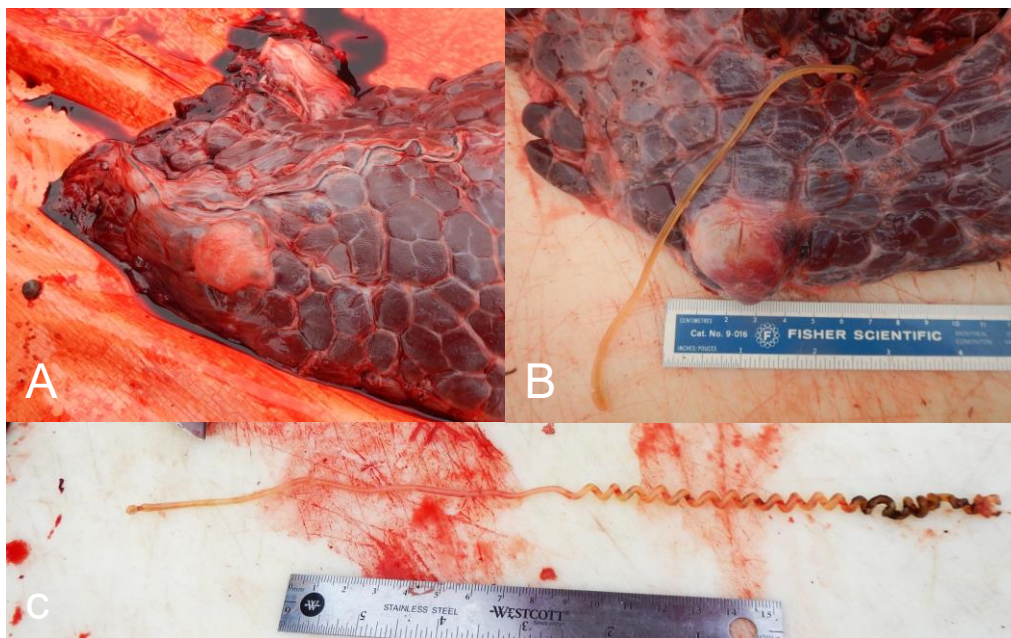


Figure 10. Renal nematodiasis in beluga whales, caused by the spirurid nematode *Crassicauda giliakiana*. Nodules formed by a cavity lined by a fibrous capsule and containing a segment of the nematode (A). Section of the renal parenchyma showing extension of the nematode further within the parenchyma (B). *Crassicauda giliakiana* with typical the cork screw appearance (C).

HEMATOPOIETIC SYSTEM

The mesenteric lymph nodes of 9 beluga whales contained firm irregular nodules (Figure 11). Similar nodules were also present in the mesentery of one animal. These nodules were characterized by a chronic inflammatory reaction usually centered on trematode eggs and/or degenerated segments of parasites. This type **granulomatous lymphadenitis associated with trematodes** has not been described in beluga whales. However, similar lesions were reported in Hector's dolphins (*Cephalorhynchus hectori*), in which it was tentatively attributed to *Campula* sp. (family Brachycladidae)

(Hutton 1987). In odontocetes, most of the digenean trematodes of the genus *Campula* are associated with the bile and pancreatic ducts (Measures 2018). The life cycle of these parasites is poorly known. In the present cases, the encapsulated lesions were unlikely to have significant impact on the health of the animals. Trematodes found in whales are not known to be harmful to people.

Other lesions associated with the lymph nodes included fibrosis (7) and multifocal aggregation of neutrophils and eosinophils (sometimes associated with Splendore-Hoeppli bodies) in the cortex or capsule (12). The focal cortical cellular infiltrations may be associated with hematogenous drainage of pathogenic organism (bacterial, parasitic) through the lymph node and is a common finding in marine mammals (Valli 2016, Simpson 1972). Increased fibrosis could hypothetically be associated with senescence, as it has been reported in human superficial inguinal nodes (Ahmadi 2013).

Accessory spleen and the presence of a splenic scar were observed in 1 individual each. The only histologic lesion noted was a microscopic, benign hemangioma in one individual.



Figure 11. Hard nodules associated with infection by trematodes in the mesenteric lymph node.

CENTRAL NERVOUS SYSTEM

Section from the *cerebrum*, *cerebellum*, and *medulla oblongata* were evaluated in all individuals except one for which only the cerebellum was available. The only changes observed were nonsignificant and compatible with changes related to age, in two individuals.

PTERYOIDES SINUSES

Nematodes found in the pteryoides sinuses (around the ear bones) had the morphology of *Pharurus pallasii*. This parasite, which is quite common in beluga whales (Wazura 1986, Measures 1995, Lair 2014), is not known to be associated with any health issue for the whale.

SURVEY FOR TOXOPLASMOSIS AND TRICHINELLOSIS

Neither larvae of *Trichinella* sp. nor cysts of *Toxoplasma* were detected during the histological examination of the tissues. However, histopathology is not a sensitive method for these parasites.

From 33 serum samples, only one from the East Whitefish harvest station (AREW15-06) was considered suspicious by ELISA and positive by IFAT for antibodies to *Toxoplasma gondii*. From 31 heart samples and 23 brain samples tested with magnetic capture DNA extraction followed by qPCR, *T. gondii* DNA was detected in only one heart sample (ARHI-DL-2015-06). Larvae of *Trichinella* were not detected in tongue and diaphragm muscles examined from belugas harvested in 2015-2016.

The detection of antibodies to *T. gondii* indicates previous exposure to the parasite and not necessarily that the animal has the infection. The cysts are microscopic, and it is also possible that cysts simply weren't present in the tissues sampled from this animal. The detection of *T. gondii* DNA in tissue indicates that the parasite is present in the organ, but it could be a very early infection, before the immune system of the animal could produce antibodies to the parasite.

CONCLUSION

This report presents the methodology and findings of two seasons of full post-mortem examinations performed on harvested beluga whales from the East Beaufort Sea population.

The whales examined during these seasons appeared to be healthy. With the exception of two animals, the beluga whales examined seemed to be in fair to excellent body condition. If we assumed that the examined animals are representative of the population, this observation suggests a good level of fitness in the East Beaufort Sea population. Even if the scaled mass index, which require the full weight of the animals and its total length, remain the gold standard to assess body condition in this species, our observations suggest that the "½ axillary girth / length" is the best alternate indices to follow body condition in this population of beluga whales. It is recommended to weight more animal to better evaluate the correlation between these alternative indices and the scaled mass index.

Most of the pathological findings detected were associated with infections by different species of parasites. The parasitic burden was low and therefore not considered to have a significant impact on the general health and fitness of the whales. This suggests that a "healthy" equilibrium between the beluga whales from this population and their parasites.

Changes observed in the endocrine system are likely incidental, age-related, findings and have been described in other beluga populations or cetacean species. Other observations generally pertained to normal physiologic processes or were age-related, such as multiple circular depressions of the skin surface, suggested to be associated with a normal molting process and reproductive senescence (menopause) in an older female.

Histopathologic examination of tissues did not reveal the presence of parasites that could potentially transmit to harvesters. *Trichinella* larvae was not detected in tongues and diaphragms; therefore, the risk of transmission of this parasite to people is very low. We did not expect to detect *Trichinella* in beluga because animals only get this parasite from eating other carnivores; however, because another marine mammal, walrus, is a known source of *Trichinella*, it was worth testing beluga.

Risk of transmission of *T. gondii* is considered low; with only 2 of 34 animals (6%) positive. Pregnant or immunocompromised people should consider freezing solid for at least 3 days, or cooking meat and organs thoroughly, to further reduce risk of transmission. For the general population and based on the animals examined, consumption of properly prepared and stored *mipku* and *muktuk* should not represent a biological risk.

The comparison between observations made during these post-mortem examinations of harvested whales with data from stranded endangered beluga whales from the St. Lawrence estuary are interesting. However, due to obvious sampling biases (harvesting of healthy individual in the Beaufort Sea compared to necropsy of dead, therefore sick animals in the St. Lawrence estuary) comparison between these two datasets should be done carefully. Nevertheless, it is obvious that sick/dead animals from the St. Lawrence estuary population have a much higher parasitic burden than the Beaufort Sea beluga whales.

It is recommended to perform this type of complete health assessment at a regular interval or in order to detect potential changes in the health of this population that could be associated with changes in the environment of these whales. The data provided annually by the whale monitors are also of great interest. These should at least include the total length, ½ circumferences and blubber thicknesses. Any anomalies of unknown origin should also be photographed, sampled and submitted for pathological analysis.

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Night sun over the camp

APPENDIX I – MORPHOLOGICAL AND MORPHOMETRIC DATA

#ARHI DL-	Sex	Age (years)	Body condition	Carcass weight (kg)	Epaxial muscle weight (kg)	Maximum width (cm)	Full length (cm)	Scaled mass index (kg)	½ Fluke width (cm)	1/2 girth (cm)			Blubber depth – (cm)			
										Axillary	Maximal	Sacral	Axillary dorsal	Ventral midline	Mid-height	Sacral
2015-02	Male	25	Good	N/A	N/A	N/A	386		50.8	101.6	121.9	61.6	N/A	10.2	7	N/A
2015-03	Male	20	Poor	595	N/A	N/A	380	609	44.4	N/A	90.2	50	N/A	3.8	N/A	6.3
2015-04	Male	32	Good	1018	35	N/A	446	595	50.8	105.4	128.3		6	8.8	6.5	5
2015-05	Male	17	Poor	564	17	N/A	353	748	44.5	94	99	51.4	5.1	7	5.1	8.3
2015-06	Female	45	Good	612	N/A	N/A	351	828	45.7	94	106.7	58.4	6.9	6.5	4.8	6
2015-08	Male	22	Fair	683	14	N/A	389	644	N/A	95.3	99.1	58.4	4.5	7.6	4.6	7.6
2015-12	Male	20	Fair	750	17	58	386	727	53.3	96.5	101.8	53.4	5	5.2	5.9	5.3
2015-13	Male	22	Good	782	25	67	401	663	N/A	94	90.2	54.6	4.1	5	4	4.7
2015-14	Female	55	Fair	N/A	10	62	346		40.6	86.4	91.5	45.7	4.8	4.3	4.5	3.8
2015-15	Female	35	Good	N/A	18	60	380		41.3	N/A	86.3	51	5.4	6.8	5	8.2
2015-16	Male	39	Excellent	693	24	66	429		55.3	107.4	110.5	52.1	4.8	4.5	4.5	8.3
2015-17	Male	25	Fair	N/A	22	58	409		52.7	97.9	111.7	54.6	6	6.5	6.2	N/A
2015-19	Male	34	Good	N/A	31	82	422		52.1	95	106	56	7	6	7	5.5
2015-20	Male	27	N/A	N/A	N/A	58	414		47	116.8	121.9	61	5	8	7	11
2015-21	Male	23	Fair	624	12	67	376	663	41.9	90	95	50	8	6.4	3.8	7
2015-22	Male	31	Excellent	N/A	30	N/A	437		51	116	115	56	6.8	7.5	6.6	8
2016-02	Female	N/A	Good	N/A	N/A	N/A	342		72*	89	96	58	6.5	7.5	5.6	N/A
2016-03	Male	N/A	Good	N/A	28	N/A	404		87*	100	115		6.2	91	7.5	5.4
2016-04	Male	N/A	N/A	N/A	N/A	N/A	434		99*	129	131	64	7	7.5	7.5	10.2
2016-05	Male	N/A	Excellent	1147	N/A	N/A	445	676	N/A	115	125	64	8.6	11	5.8	7
2016-06	Male	N/A	Good	N/A	25	74	424		109*	117	134	64	8.5	12	5.5	6.2
2016-07	Male	N/A	Excellent	1058	29	86	411	823	102*	121	143	69	7.5	12.9	9.4	8
2016-08	Male	N/A	Good	N/A	29	74.5	432		104*	N/A	109	68	7.5	11.4	7.2	7.5
2016-09	Male	N/A	Good	N/A	28	59	427		95*	122	135	70	6	4.5	4	5
2016-10	Male	N/A	Good	N/A	25	59.5	399		96*	113.5	111	70.5	57	9	5.5	7.4
2016-11	Male	N/A	Excellent	N/A	31	70	412		105*	105	131	64	8	7	7	4.5

*In 2016, the complete fluke width was measured rather than ½ width.

APPENDIX II – NECROPSY PROTOCOL - 2015

Beluga health assessment 2015 / Necropsy protocol Hendrickson Island

I. SAMPLING

1. General (unless otherwise specified)

- Histology sample; 2x2x1 cm
- Sample to be frozen; 1x2x4 cm, -20°C
- Toxicology samples: beluga monitor collects these samples using standardized sampling kit
- Ecology samples: use fresh scalpel blade when sub-sectioning tissue from different animals

II. EXTERNAL EXAMINATION

1. Photographs – standard

- Complete left and right laterals
- Rostro-caudal view
- Caudo-rostral view (make sure whole length of the beluga is visible in the picture)



2. Standard sampling

- Normal skin for histology
- Normal skin and blubber, just cranial to dorsal crest, 25 g x 2 (-20°C, Whirlpack) - Thyroid hormone assessment (S. Lair)
- If a lactating female has been harvested, collect milk in a plastic plastic tube (15 ml) and freeze (-20°C)
- Collect fat for toxicology above the flipper, at midline (Monitor)
- Collect blubber subsample for cortisol (L. Loseto)
- Genetics sampling: collect skin and place in buffer (Monitor)



3. External lesions/ sampling

- Photograph any external lesions, with ruler inserted in the picture + animal ID
- Describe any evidence of trauma other than related to the hunting method as well as pox lesions (pinpoint holes to rough mottled areas) or herpesvirus lesions (punched out ulcers or erosions)
- Sample external lesions of interest for histology + freezing (-20°C)
- If herpesvirus associated lesions suspected, swab (Ole N.) (-80°C) and histology sample (S. Lair) to be collected
- Collect Fecal and urogenital swabs: cryovial (Ole N.) (- 80°C)

III. MEASUREMENTS

1. Carcass weight

- Record the net and gear weight before weighing the beluga's carcass as a whole (kg)

2. Standard measurements (refer to diagram below) * With whale monitors

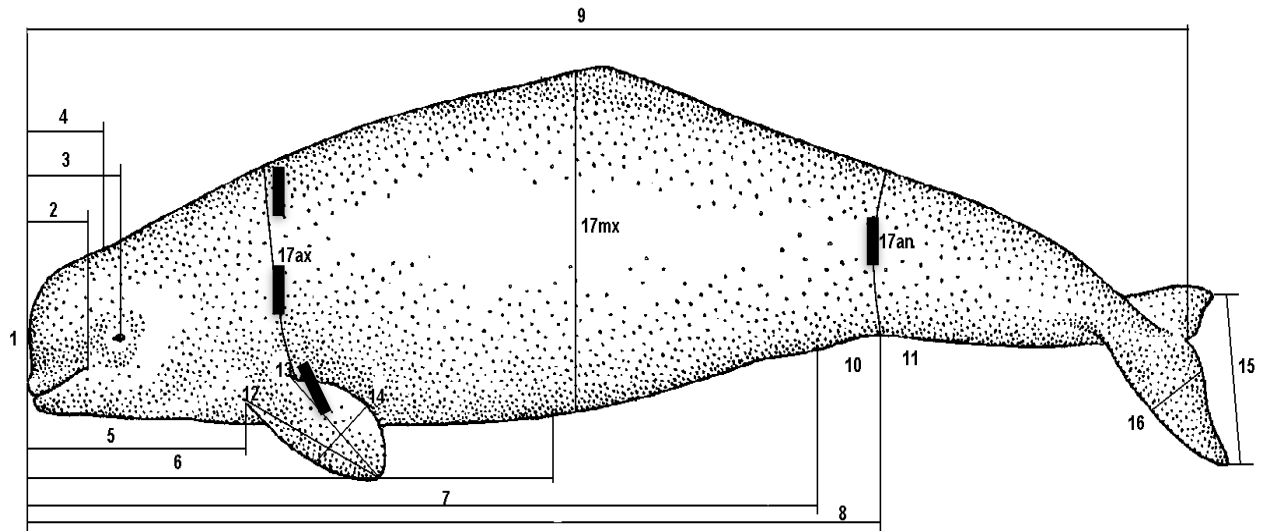
- Maximum width (whale in ventral recumbency)
- Full length (horizontal) – 9
- ½ Fluke width – central indentation up to the tip of the fluke
- ½ Axillary girth – 17ax
- ½ Maximal girth – 17mx
- ½ Sacral girth – 17an
- Blubber depths – thick lines

- Measure from muscle layer to intersection between dermis and epidermis
- Axillary (dorsal midline, ventral midline, halfway)
- Dorsosacral (close to dorsal midline at the level of the anus)

3. At this point, muktuk and meat may be collected

4. Epaxial muscle weight

- If possible: remove and weigh the entire epaxial muscle mass from L1-L12.



IV. INTERNAL EXAMINATION AND SAMPLING

1. Blood sampling

- Through a heart / aorta puncture (preferred) or tail / neck vessel transection (avoid salt water / blubber contamination), fill the following tubes with blood;
 - Red top tubes, 10 ml (serum): five tubes
 - Green top tubes (Heparin), 10 ml (plasma): three tubes
- Keep samples at 4-5°C until centrifugation.

2. Musculoskeletal

- Note the number of teeth for each quadrant (maxillary / mandibular, upper/ lower), and note amount of wear
- Remove lower mandible with a hacksaw (aging)
- Open both shoulder joints and note any abnormality.
 - Freeze joint fluid if of abnormal appearance.
- Sample epaxial muscle for histology
- Sample longitidimus dorsus for toxicology (Monitor)
- Toxoplasma related samples (E. Jenkins)
 - Skeletal muscle (epaxial muscle), 100 g (Whirlpack, -20°C) – magnetic capture PCR
 - Skeletal muscle, small sample in cryovial (-80°C) – traditional PCR
- Trichinella related samples (E. Jenkins)
 - Muscles of the tongue, 100 g (Whirlpack, -20°C)
 - Diaphragm, 50 g (Whirlpack, -20°C)

- Active skeletal muscle, 50 g (Whirlpack, -20°C)

3. Head examination

- Thyroid
 - Weigh thyroid gland once excess tissue is removed
 - Sample for histology + freezing (100 g, -20°C, Whirlpack)
- If possible: Remove ear bones (UBC-Maria Morrell) before splitting skull open
- Slit the skull open with the axe
 - Collect the following samples for histology + freezing
 - Cerebrum (x2)
 - Cerebellum (x1)
 - Cerebral trunk (x1)
 - Toxoplasma related samples
 - Brain, 100 g (Whirlpack, -20°C) – magnetic capture PCR
 - Brain, small sample in cryovial (-80°C) – traditional PCR
 - Pituitary gland
 - Fixed whole in formalin

4. Open the body cavities

- Transect the ventral and dorsal aspects of the right costal arch with cutters
- Note any abnormality non-related to the harvesting method and sample abnormal fluid for freezing (-20°C, 5ml cryovial)
- **Note:** if the carcass must be removed quickly from the beach, harvest all organs and keep on a plastic tarp until they can be examined

5. Adrenal glands

- Fix both adrenal glands in formalin

6. Respiratory

- Collect a swab from the blowhole and place in buffer solution, at -80 (Ole)
- Collect one superficial (with pleura), one median and one deep (with at least one bronchi) parenchyma sample from each of the cranial, median and caudal portion of each lobe (total of 18 samples)
- For each lung section, place the three collected samples in a same white histology cassette. Always keep the same order between samples to facilitate further analysis.
- Collect a sample from each lung for freezing (Whirlpack, -20°C)

7. Cardiovascular

- Examine all 4 heart chambers and valves
- Collect one sample (IVS) for histology and freezing
- Toxoplasma related samples (E. Jenkins)
 - Heart, 100 g (Whirlpack, -20°C) – magnetic capture PCR
 - Heart, small sample in cryovial (-80°C) – traditional PCR

8. Hepatobiliary

- Bread loaf the liver
- Sample for histology and freezing
- Collect two liver pieces for toxicology
- Collect liver sample for toxoplasma (2 ml cryovial, -80°C)
- Collect bile for toxicology

9. Lymphoid

- Collect one sample of each of the following for histology and freezing
 - Spleen
 - Tracheobronchic, mediastinal and mesenteric lymph nodes
 - Thymus if present
 - Collect spleen sample for toxoplasma (2 ml cryovial, -80°C)

10. Urinary

- Bread loaf the kidneys
- Collect samples for histology and freezing from the bladder and each kidney
- Collect urine sample for toxicology

11. Digestive system

- Look through the digestive tract for lesions / parasites and examine content
- Sample pancreas for histology
- Sample duodenum for histology
- Collect
 - GIT trematodes (*Synthesium seymouri*), 5-10 in (70% ethanol, plastic tube 15ml) (Mariana Ebert)

12. Reproductive

- Collect a sample from each testicle and epididymis for histology
- Put both whole ovaries in formalin for histology.
- If a foetus is present, record length and sex. Note any abnormality
- Mammary gland; Collect samples for histology and freezing

13. Processing of blood samples

- Keep sample at 4-5°C until centrifugation (3000 rpm, 10 min). Collect and freeze (-80°C) the serum.
- Serum will be used for (separate in 2 ml cryovials):
 - Toxoplasma MAT serology; 3 ml of serum
 - Brucella – serology (L. Losetto); 3ml
 - Morbillivirus – serology (L. Losetto); 3 ml
 - T3-T4 – dosing (S. Lair); 3 ml of serum
 - Cortisol: 2 x 500 ul plasma

APPENDIX III – NECROPSY SAMPLING LIST 2015

GENERAL SAMPLING GUIDELINE (UNLESS OTHERWISE SPECIFIED)

- Histology sample; 2x2x1 cm in formol
- Sample to be frozen; 1x2x4 cm, -20°C in Whirlpack
- Name additional lesion-related samples and add them under the according system's checklist. Refer to this sample's name when fully describing the lesion at the end of the section

EXTERNAL EXAMINATION**Photographs**

Complete left lateral	<input type="checkbox"/>
Complete right lateral	<input type="checkbox"/>
Rostro-caudal view	<input type="checkbox"/>
Caudo-rostral view	<input type="checkbox"/>
Skin lesions?	<input type="checkbox"/>

Standard sampling – Skin and Blubber

Sample	Collected?	Detail	Conservation	User / Usage
Skin & blubber	<input type="checkbox"/>		Formol	CWHC / Histology
Skin	<input type="checkbox"/> <input type="checkbox"/>	25g X 2	Medium Whirlpack. -20°C. *	CWHC / Thyroid assessment
Milk	<input type="checkbox"/>	15 ml	Plastic tube, 15 ml. -20	CWHC
Blubber	<input type="checkbox"/>			DFO / toxicology
Blubber	<input type="checkbox"/>			L. Losetto / cortisol
Skin	<input type="checkbox"/>		buffer	DFO / Genetics
Swabs	<input type="checkbox"/>	Fecal urogenital /	In cryovial, -80°C	Ole N.

Additional samples (note, swab (-80°C) + histo sample if herpesvirus suspected – Ole N.):

Notes

Lesions: Name sample, describe lesions and collect for histology and freezing. Use verso if needed.

MEASUREMENTS

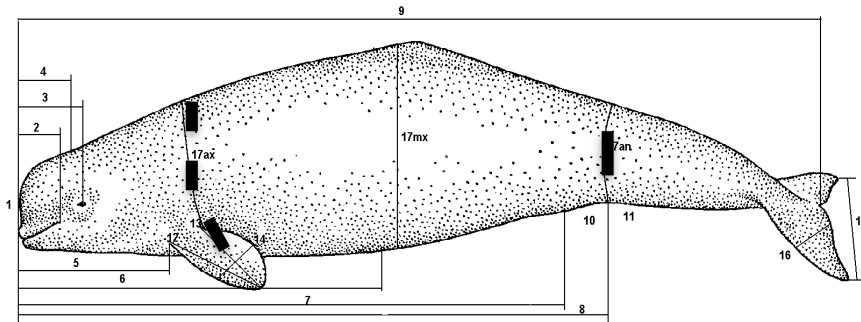
Carcass weight (kg):

Epaxial muscle weight:

Standard measurements:

- Maximum width (dorso-ventral)
- 9 Full length (horizontal)
- 15 Fluke width
- 17ax ½ Axillary girth
- 17mx ½ Maximal girth
- 17an ½ Sacral (anal) girth
- Blubber depth – Axillary, dorsal
- Blubber depth – Axillary, ventral midline
- Blubber depth – Axillary mid-height
- Blubber depth – Sacrodorsal, anus level

cm



Additional notes:

INTERNAL EXAMINATION AND SAMPLING

1. Blood

- Collection --> Time: Location:
- Red top tubes (x5)
- Green top tubes (x3)
- Note on conservation:

2. Musculoskeletal

- Teeth wear:

Quadrant	Number of teeth	Quadrant	Number of teeth
Maxillary L		Mandibular L	
Maxillary R		Mandibular R	

Collection

Sample	Collected?	Detail	Conservation	User / Usage
Mandible	<input type="checkbox"/>	Hemi -mandible	Ziploc bag -20°C	CWHC
Synovial fluid	<input type="checkbox"/> <input type="checkbox"/>	*If abnormal appearance, 5 ml	5 ml cryovial, -20°C	CWHC / bacteriology
Epaxial muscle	<input type="checkbox"/>		Formol	CWHC / histology

Sample	Collected?	Detail	Conservation	User / Usage
<i>Longitimus dorsus m.</i>				DFO / Toxicology
Skeletal muscle	<input type="checkbox"/>	100g, active swim muscle	Whirlpack, -20°C	E. Jenkins / Toxoplasma
Skeletal muscle	<input type="checkbox"/>	Small sample	2 ml Cryovial, -80°C	E. Jenkins / Toxoplasma
Muscular tongue	<input type="checkbox"/>	100 g	Whirlpack, -20°C	E. Jenkins / Trichinella
Diaphragm	<input type="checkbox"/>	50 g	Whirlpack, -20°C	E. Jenkins / Trichinella
Skeletal muscle	<input type="checkbox"/>	50 g	Whirlpack, -20°C	E. Jenkins / Trichinella

Additional samples:

Notes

Lesions: Name sample, describe lesions and collect for histology and freezing. Use verso if needed.

3. Head examination

- Thyroid weight (g):

Collection

Sample	Collected?	Detail	Conservation	User / Usage
Thyroid	<input type="checkbox"/>		Formol	CWHC / Histology
Thyroid	<input type="checkbox"/>	100g	Whirlpack, -20°C	CWHC
Ear bones	L R <input type="checkbox"/> <input type="checkbox"/>			UBC / Maria Morrell
Cerebrum	<input type="checkbox"/> <input type="checkbox"/>		Formol	CWHC / Histology
Cerebellum	<input type="checkbox"/>		Formol	CWHC / Histology
Cerebral trunk	<input type="checkbox"/>		Formol	CWHC / Histology
Cerebrum / cerebellum / cerebral trunk	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>		Whirlpack, -20°C	CWHC
Cerebrum	<input type="checkbox"/>	100g	Whirlpack, -20°C	E. Jenkins / Toxoplasma
Cerebrum	<input type="checkbox"/>	Small sample	2 ml Cryovial, -80°C	E. Jenkins / Toxoplasma
Pituitary gland	<input type="checkbox"/>	Whole	Formol	CWHC

Additional samples:

4. Body cavities

5. Adrenal glands

- Adrenal gland weight (g): Left: Right:

Collection

Sample	Collected?	Detail	Conservation	User / Usage
Adrenal glands	L R <input type="checkbox"/> <input type="checkbox"/>	Whole	Formol	CWHC / Histology

6. Respiratory*Collection*

Sample	Collected?	Detail			Conservation			User / Usage
Blowhole	<input type="checkbox"/>	Swab			Buffer solution, -80°C			
Lung	LR <input type="checkbox"/> <input type="checkbox"/>				Whirlpack, -20°C			CWHC
Lung	<input type="checkbox"/>	See table below			Each set of three in same white histology cassette.			CWHC / histology
Lung : right	superf	median	deep	Lung : left	superf	median	deep	
cranial	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	cranial	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
medial	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	medial	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
caudal	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	caudal	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

*Additional samples:***7. Cardiovascular***Collection*

Sample	Collected?	Detail	Conservation	User / Usage
Interventricular septum	<input type="checkbox"/>		Formol	CWHC / Histology
Interventricular septum	<input type="checkbox"/>		Whirlpack, -20°C	CWHC
Heart	<input type="checkbox"/>	100g	Whirlpack, -20°C	E. Jenkins / Toxoplasma
Heart	<input type="checkbox"/>	Small sample	2 ml Cryovial, -80°C	E. Jenkins / Toxoplasma

*Additional samples:***8. Hepatobiliary***Collection*

Sample	Collected?	Detail	Conservation	User / Usage
Liver	<input type="checkbox"/>		Formol	CWHC / Histology
Liver	<input type="checkbox"/>		Whirlpack, -20°C	CWHC
Liver	<input type="checkbox"/>		2 ml Cryovial, -80°C	E. Jenkins / Toxoplasma

*Additional samples:***9. Lymphoid***Collection*

Sample	Collected?	Detail	Conservation	User / Usage
Spleen	<input type="checkbox"/>		Formol	CWHC / Histology
Spleen	<input type="checkbox"/>		Whirlpack, -20°C	CWHC
Spleen	<input type="checkbox"/>		2 ml Cryovial, -80°C	E. Jenkins / Toxoplasma
Lymph nodes	T Md Ms <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	T; tracheobronchic Md: mediastinal Ms: mesenteric	Formol	CWHC / Histology
Lymph nodes	T Md Ms <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>		Whirlpack, -20°C	CWHC

Thymus	<input type="checkbox"/>		Formol	CWHC / Histology
Thymus	<input type="checkbox"/>		Whirlpack, -20°C	CWHC

Additional samples:

10. Urinary Collection

Sample	Collected?	Detail	Conservation	User / Usage
Bladder	<input type="checkbox"/>		Formol	CWHC / Histology
Bladder	<input type="checkbox"/>		Whirlpack, -20°C	CWHC
Kidney	<input type="checkbox"/>		Formol	CWHC / Histology
Kidney	<input type="checkbox"/>		Whirlpack, -20°C	CWHC
Urine				DFO / Toxicology

Additional samples:

11. Digestive system Collection

Sample	Collected?	Detail	Conservation	User / Usage
Trematodes	<input type="checkbox"/>	<i>Synthesium seymouri</i>	70% Ethanol, 15 ml plastic tube	Mariana Ebert
Feces	<input type="checkbox"/>	100g	Whirlpack, -20°C	CWHC / Thyroid assessment
Feces	<input type="checkbox"/>	7ml	15 ml plastic tube, little bit formol	CWHC / coprology

12. Reproductive Collection

Sample	Collected?	Detail	Conservation	User / Usage
Testicle	LR <input type="checkbox"/> <input type="checkbox"/>		Formol	CWHC / Histology
Epididymis	LR <input type="checkbox"/> <input type="checkbox"/>		Formol	CWHC / Histology
Ovaries	LR <input type="checkbox"/> <input type="checkbox"/>	Whole	Formol	CWHC / Histology

Additional samples:

Notes

Lesions: Name sample, describe lesions and collect for histology and freezing. Use verso if needed.

APPENDIX IV – NECROPSY PROTOCOL 2016

Beluga health assessment 2016

Necropsy protocol Hendrickson Island

V. SAMPLING

1. Permissions

- *Meet hunters with whale monitor*
- *Always ask hunters if the whale can be sampled*
- *Ask hunters if pictures can be taken during sampling. We are preparing a sampling guide to help with stranded whales. The more pictures, the more helpful it will be ☺*

2. General (unless otherwise specified)

- Histology sample; 2x2x1 cm
- Sample to be frozen; 1x2x4 cm, -20°C
- Toxicology samples: beluga monitor collects these samples using standardized sampling kit
- Ecology samples: use fresh scalpel blade when sub-sectioning tissue from different animals
- Sample any lesion of interest for histology + freezing (-20°C)
- Pictures: Photograph any lesion and make sure a ruler is in the picture. Always make sure the identification of the whale (ARHI-DL-2016-**) is present on the picture of an animal.

3. Precautions

- Always be aware of the other persons around when handling a knife or other sharps to avoid accidents.
- Never lay down a sharp instrument in the carcass or the water (risk of injury) and avoid the sand (makes them blunt).
- Knives labelled with a red tape should be reserved for internal organs.
- When multiple whales are to be sampled in a short time frame, proceed to external examination, measurements, blood sampling, removal of adrenal glands, thyroid and spleen. Remove all internal organs and place them in an identified Rubbermaid box and keep the head aside for later subsampling.
- Avoid contact with sand and internal organs for every tissue destined for human consumption (blubber and skin, meat).
- Avoid contact of organs destined for subsampling with sand (as much as possible).
- Keep tissue remains together so that they can be discarded easily at the end of the necropsy without offering the gulls a buffet.
- Keep Whirl-PAKs clean when tissues are inserted. Remove air before closing the bag.

VI. EXTERNAL EXAMINATION

1. Photographs – standard

- Complete left and right laterals
- front to back view
- back to front view

Make sure whole length of the beluga is visible in all pictures



2. Standard sampling

- Normal skin for histology
- Normal skin and blubber, ventral to dorsal crest, 25 g x 2 (-20°C, Whirlpack) - Thyroid hormone assessment (S. Lair)
- If a lactating female has been harvested, collect milk in a plastic tube (15 ml) and freeze (-20°C). Collect mammary gland for histology and freezing.
- Collect fat for toxicology (fist size) above the flipper, at midline (Monitor)
- Collect a whole depth skin and blubber sample (same region as for toxico) for picture (with color scale) and subsample for cortisol/hormones (skin, inner and outer blubber in cryovials at -80°C) (L. Loseto)
- Collect a small piece of inner blubber in RNA later and place at room temperature for 24h before freezing (-20°C)
- Genetics sampling: collect skin and place in buffer (Monitor)

3. External lesions/ sampling

- Describe any evidence of trauma other than related to the hunting method as well as pox lesions (pinpoint holes to rough mottled areas) or herpesvirus lesions (punched out ulcers or erosions)
- If herpesvirus associated lesions suspected, swab (Ole N.) (-80°C) and histology sample (S. Lair) to be collected
- Collect skin, blowhole, oral, fecal and urogenital swabs: cryovial (Ole N.) (- 80°C)

VII. MEASUREMENTS

1. Carcass weigh

- *Ask hunters if the whale can be weighed (Objective: 15 min duration)*
- Record the net and gear weight before weighing the beluga's carcass as a whole (kg)

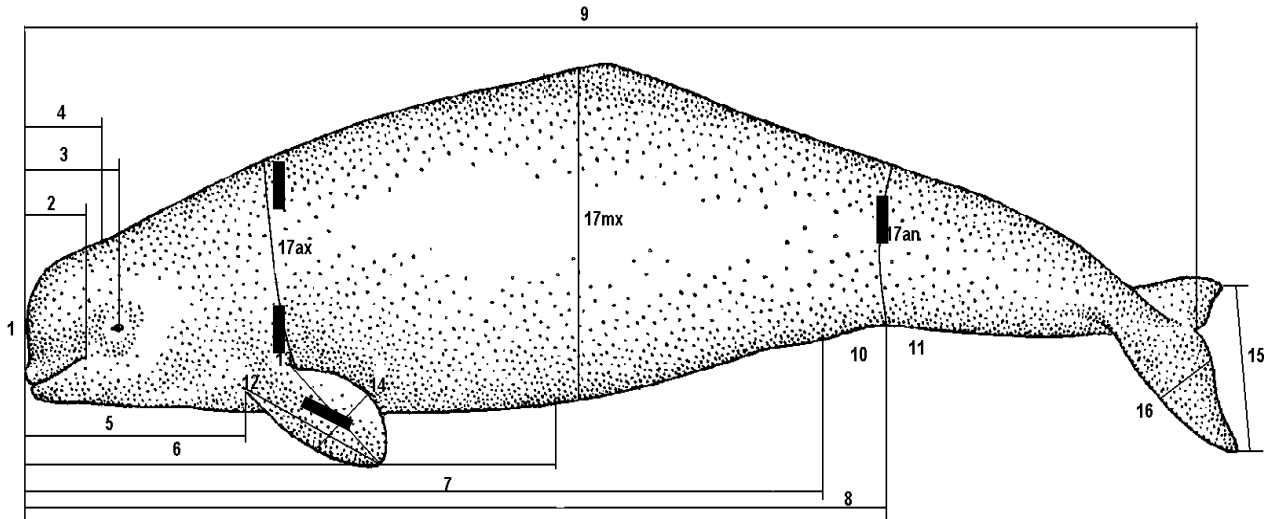
2. Standard measurements (refer to diagram below) * With whale monitors

- *Take all measurements in cm*
- Maximum width (whale in ventral recumbency)
- Full length (horizontal) – 9
- Fluke width – 15
- ½ Axillary girth – 17ax
- ½ Maximal girth – 17mx
- ½ Sacral (anal) girth - 17an
- Blubber depths – thick lines
 - Double full thickness transverse blubber incision; measure from muscle layer to intersection between dermis and epidermis
 - Axillary (dorsal midline, ventral midline, halfway)
 - Sacral (close to dorsal midline at the level of the anus)

3. At this point, muktuk and meat may be collected

4. Epaxial muscle weight

- Ask hunters if the meat can be weighed
- If possible: remove and weigh the entire epaxial muscle mass from L1-L12 in a clean IKEA bag.



VIII. INTERNAL EXAMINATION AND SAMPLING

1. Open the body cavities

- Wait until the second side of beluga has been flensed before opening abdomen and thorax.
- Transect the ventral and dorsal aspects of the right costal arch with cutters
- Note any abnormality non-related to the harvesting method and sample abnormal fluid for freezing (-20°C, 5ml cryovial)
- **Note:** if the carcass must be removed quickly from the beach, harvest all organs and keep in a rubbermaid container until they can be examined

2. Blood sampling

- *With the whale monitor:*
- Through a heart / aorta puncture (preferred) or tail / neck vessel transection (avoid salt water / blubber contamination), fill the following tubes with blood;
 - Red top tubes, 10 ml (serum): five tubes
 - Green top tubes (Heparin), 10 ml (plasma): three tubes
 - Whale monitor's blood tube
 - RNAlater blood tube (room temperature 2h then freeze at -20°C)
- Gently inverse blood tubes a few times. Red top and green top tubes should be allowed to sit for minimum 15 minutes before centrifugation. After 15 minutes, tubes can be put in a cooler with ice packs if time before centrifugation is to be longer.

3. Musculoskeletal

- Note the number of teeth for each quadrant (maxillary / mandibular, upper/ lower), and note amount of wear
- Remove lower mandible with a hacksaw (monitor, aging)
- Open both shoulder joints and note any abnormality.
 - Freeze joint fluid if of abnormal appearance.

- Sample epaxial muscle for histology and freezing
- Toxoplasma related samples (E. Jenkins)
 - Skeletal muscle (epaxial muscle), 100 g (Whirlpack, -20°C) – magnetic capture PCR
 - Skeletal muscle, small sample in cryovial (-80°C) – traditional PCR
- Trichinella related samples (E. Jenkins)
 - Muscular tongue, 100 g (Whirlpack, -20°C)
 - Diaphragm, 50 g (Whirlpack, -20°C)
 - Active skeletal muscle, 50 g (Whirlpack, -20°C)
- Collect one large piece of epaxial muscle (Monitor - toxicology)

4. Adrenal glands

- Weigh each adrenal glands
- Fix both adrenal glands in formalin

5. Respiratory

- Collect one superficial (with pleura), one median and one deep (with at least one bronchi) parenchyma sample from each of the cranial, median and caudal portion of each lobe (total of 18 samples)
- For each lung section, place the three collected samples in a same white histology cassette. Always keep the same order between samples to facilitate further analysis.
- Collect a sample from each lung for freezing (Whirlpack, -20°C)

6. Cardiovascular

- Examine all 4 heart chambers and valves
- Collect one sample (interventricular septum) for histology and freezing
- Toxoplasma related samples (E. Jenkins)
 - Heart, 100 g (Whirlpack, -20°C) – magnetic capture PCR
 - Heart, small sample in cryovial (-80°C) – traditional PCR

7. Hepatobiliary

- Bread loaf the liver
- Sample for histology and freezing
- Collect two liver pieces for toxicology (Monitor and L.Loseto)
- Collect liver sample for toxoplasma (2 mL cryovial)
- Collect a small liver sample in RNA later and place at room temperature for 24h before freezing (-20°C)
- Collect bile for toxicology

8. Lymphoid

- Spleen
 - Sample for histology and freezing
- Collect spleen sample for toxoplasma (2 ml cryovial, -80°C)
- Tracheobronchic, mediastinal and mesenteric lymph nodes
 - Sample for histology and freezing
 - If granulomas are noted in mesenteric lymph nodes, cut them in half and put ½ in ethanol 70% and freeze the other half. Keep some granulomas for histology.
- Collect thymus for histology if present

9. Urinary

- Bread loaf the kidney
- Collect samples for histology and freezing from the bladder and each kidney
- Collect urine sample for toxicology

10. Digestive system

- Look through the digestive tract for lesions / parasites and examine content
- Sample pancreas for histology

- Sample duodenum for histology
- Collect
 - GIT trematodes (*Synthesium seymouri*), 5-10 in (70% ethanol, plastic tube 15ml) (Mariana Ebert)
 - Feces, 100 g (-20°C, Whirlpack) – Thyroid hormone assessment (S. Lair)
 - Feces, 7 ml (15 ml plastic tube with enough formalin to obtain thick but fluid consistency) – coprology (S. Lair)

11. Reproductive

- Collect a sample from each testicle and epididymis for histology
- Put both whole ovaries in formalin for histology.
- If a foetus is present, record length and sex. Note any abnormality

12. Head examination

- Eye collection (Monitors)
- Thyroid
 - Weigh thyroid gland once excess tissue is removed
 - Sample for histology + freezing (100 g, -20°C, Whirlpack)
- Slit the skull open with the axe
 - Collect the following samples for histology + freezing
 - Cerebrum (x2)
 - Cerebellum (x1)
 - Cerebral trunk (x1)
 - Toxoplasma related samples
 - Brain, 100 g (Whirlpack, -20°C) – magnetic capture PCR
 - Brain, small sample in cryovial (-80°C) – traditional PCR
 - Pituitary gland
 - Fixed whole in formalin

13. Processing of blood samples

- Keep sample at 4-5°C until centrifugation (3000 rpm, 10 min). Collect and freeze (-80°C) the serum.
- *If blood does not appear to separate, spin an additional 10 min. Please note that plasma or serum may be dark red but should still be collected. If separation issues occur, discuss next steps with Emilie Couture and DO NOT DISCARD.*
- Serum will be used for (separate in 2 ml cryovials):
 - Toxoplasma MAT serology; 2 ml of serum
 - Brucella – serology (L. Losetto); 2 ml
 - Morbillivirus – serology (L. Losetto); 2 ml
 - T3-T4 – dosing (S. Lair); 3 ml of serum
 - Cortisol: 2 x 500 ul plasma

14. Post sampling tasks

- The beach should be cleaned up following each sampling period. Tissue remains and carcass need to be carried away further in the water (ideally by boat).
- Necropsy knives and other instruments should be washed in seawater following sampling. Thoroughly clean IKEA bags as well.
- Protect hoist chain by storing it in appropriate bag between whales. Avoid chain contact with sand.

APPENDIX V - NECROPSY SAMPLING LIST 2016

Beluga health assessment 2016
Field checklist Hendrickson Island
 Male / Female

ARHI-DL-2016-_____
 Date: 2016-07-_____

IX. EXTERNAL EXAMINATION**1. Photographs**

Left + right lateral			Front to back		Back to front	
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2. Sampling

Skin&Blubber (histo)									
Skin&Blubber (-20) x2									
Skin (Scintillation - 20)									
Blubber (Toxico, fist size -20)									
Blubber (full depth 2x2)		Picture		Skin		Outer b.		Inner b.	
Blubber (inner, 1x1x1 RNAlater)									
Mammary gland	Histo		-20		Milk (-20)				
Swabs	Blowhole		Urogenital		Fecal		Oral		Skin

3. Notes /Additional samples**X. MEASUREMENTS**

Weights	
Carcass	kg
Muscle (L1-L12)	kg
Thyroid	g
Left adrenal	g
Right Adrenal	g

Teeth number			
Lt upper		Rt upper	

Lt lower		Rt lower	
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Lengths (all in cm)	
Maximum width	
Full length	
Fluke width	
½ Axillary girth	
½ Maximal girth	
½ Sacral (anal) girth	
Ax blubber – dorsal	

Ax blubber – 1/2	
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Ax blubber – ventral	
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*Monitor keeps lower jaw

1. Notes

XI. INTERNAL EXAMINATION AND SAMPLING

1. Blood

Red top (5)		Green top (3)		Monitor (1)		RNAlater (1)	
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2. Musculoskeletal

- Check shoulder joints

Back muscle (histo)	
B.muscle (-20:vet)	
B.muscle (100g: Toxo)	
B.muscle (cryovial: Toxo)	

B.muscle (toxico)	
B.muscle (50g Trichinella)	
Diaphragm (50 g: trichinella)	
Tongue (100g: trichinella)	

3. Head

Cerebrum x2	Histo			-20		
Cerebellum	Histo			-20		
Cerebral trunk	Histo			-20		
Brain (Toxo)	-80			100g, -20		

4. Glands/Immune system

Thyroid	Histo				-20				
Pituitary (whole, histo)				Thymus					
Adrenals (whole, histo)		L:			R:				
Spleen	Histo (H)			-20		Toxo (-80)			
L. nodes	Tb	H:	-20:	Med	H:	-20:	Mes	H:	-20:
L.N. granuloma	Histo			½ F, ½ ethanol					

5. Thorax

Heart (septum)	Histo			-20	
Heart (Toxo)	-80			100g, -20	
Lungs	Histo (x18)			-20 (x2)	

6. Abdomen

Liver	Histo			-20	
Liver (Toxo)	-80				
Liver (toxico)	-20 x2			Bile, -20	
Liver (RNA later)					
Kidney (1-2)	Histo			-20	
Bladder	Histo			-20	
Urine					
GIT trematodes	Ethanol				
Pancreas (histo)				Duodenum (histo)	
Feces (100g)	-20			7ml+ formol	
Testes (hist)				Epididymis (h)	
Ovaries (hist-whole)					

APPENDIX VI- NECROPSY REPORTS



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-02
PATHOLOGY NO.: P2462-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested of the southeast point of Hendrickson Island in Kugmallit Bay on 2015-07-10. It was from a group of approximately 16 white whales. The group appeared to be healthy and may have been feeding prior to hunt. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Muscle sarcocystosis**
2. **Mild verminous bronchopneumonia (*Halocercus* sp. suspected)**
3. **Inactive spermatogenesis**
4. **Adrenocortical cysts**
5. **Hypophyseal cysts**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. The body condition of this mature male could not be properly assessed from the pictures taken upon harvest, but love handles and fair blubber depth recorded suggested a good body condition.

The species of sarcocysts found in the muscles is unknown, although we are looking to pursue additional diagnostic. Only two species are known to infect humans through the consumption of meat. They are found in beef and pork and may cause digestive signs (nausea, vomiting enteritis), although most infected persons will show mild or no signs at all. While freezing or cooking of the meat has been shown to kill

sarcocysts, there is no reports of *Sarcocystis* species from marine mammals being harmful to humans (Fayer 2015).

The lung lesions observed in this individual were mild and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal. The predominant mononuclear cells in conjunction with some eosinophils may indicate a chronic parasitic infection. Degenerate proteinaceous material may represent degenerate parasites and the size of the structure suggests they could be attributed to *Halocercus* sp. *Halocercus monoceris*, *H. taurica* and *Stenurus arctomarinus*, are the most frequent cause of parasitic pneumonia in Saint-Lawrence beluga whales (Lair 2016).

The scarce presence of spermatozoids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

Adrenocortical cysts have been described in Saint-Lawrence beluga whales and are thought to be associated with normal aging process, although they have also been suggested to be associated with the impact of stress or adrenocorticolytic xenobiotics (Lair 1997).

Hypophyseal cysts containing colloid were a prominent feature in most cetaceans examined in a study of pathological features of odontocete cetaceans (Cowan 2008) and this observation is not considered to be significative.

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

- Cowan DF, Haubold EM, Tajima Y. Histological, immunohistochemical and pathological features of the pituitary gland of odontocete cetaceans from the western Gulf of Mexico. *Journal of Comparative Pathology*. 2008 Aug 1;139(2-3):67-80.
- Fayer R, Esposito DH, Dubey JP. Human infections with *Sarcocystis* species. *Clinical Microbiology Reviews*. 2015 Apr 1;28(2):295-311.
- Lair S, Beland P, De Guise S, Martineau D. Adrenal hyperplastic and degenerative changes in beluga whales. *Journal of Wildlife Diseases*. 1997 Jul;33(3):430-7.
- Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. *Veterinary Pathology*. 2016 Jan;53(1):22-36.
- O'Brien JK, Steinman KJ, Schmitt T, Robeck TR. Semen collection, characterisation and artificial insemination in the beluga (*Delphinapterus leucas*) using liquid-stored spermatozoa. *Reproduction, Fertility and Development*. 2008 Oct 6;20(7):770-83.
- Stewart BE, Stewart RE. *Delphinapterus leucas*. *Mammalian Species*. 1989; (336): 1-8.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture on Hendrickson Island, 2015-07-10.
Sex: Male

Age: 25 years old

Weight: ND

Body score (subjective): good (love handles reported by monitors, fair blubber depth)

Length: 386.2 cm

Carcass condition score: 2

External examination

An adult male, white in coloration is examined. Multifocal, slightly depressed circular lesions with an irregular surface are noted mainly on the cranial dorsum. Approximately 100 of those lesions were seen and their size ranged between 1 and 2 cm in diameter.

Internal examination

Spleen: Diffuse white scars of an irregular borders are noted on the serosa.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable lesions; shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, lungs, heart, liver, lymph nodes (tracheobronchic, mesenteric), urinary system (bladder, kidney), reproductive tract (testes and epididymis).

HISTOPATHOLOGY

Thyroid (B, C): Follicles are homogenous in size and are lined by an active cuboidal epithelium.

Testes (B): A small number of sperm noted in the lumen of the seminiferous tubules.

Lymph node (B): Adequate cellularity is noted. Medullary sinusoids are dilated.

Muscle (C): Cyst-like structures are noted in a few muscular fibers and contain numerous small and elongated basophil structures (sarcocysts).

Lungs (D to I): Thirteen sections had no significant changes, while the following lesions occupied up to 5% of the remaining 5 sections. Mild inflammatory changes are noted within the lungs parenchyma. Lesions consisted in rare, focal interstitial accumulation of inflammatory cells, mostly mononuclear inflammatory cells (histiocytes) as well as scarce eosinophils (D, G, H). Tubular arrangements of granular proteinaceous material may be seen in association with this reaction and are associated with multinucleated cells or accumulation of neutrophils (G, H). Focal areas of alveolar, bronchiolar and interstitial inflammatory cell accumulation are also noted in the caudal left lung (macrophages and eosinophils) (I).

Hypophysis (J): Cystic structures lined with a cuboidal epithelium are noted in the adenohiphysis (pars distalis) and form small acinic aggregates.

Adrenals (M to R): A total of 7 cortical cysts are noted. Some cysts are clustered and the total diameter ranges between 0.36 to 0.52 mm. In some, surrounding vacuolar degeneration and characteristic spider web pattern of the cystic clusterings are noted (N, Q, R).

No significant lesions on the examined sections from: heart (A), bladder (A), liver (B), spleen (B), kidney (C), central nervous system (J,K)

Histomorphologic diagnostics

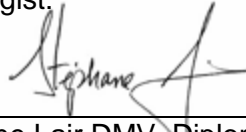
- Focal muscular sarcocystosis
- Mild, multifocal verminous bronchopneumonia associated with degenerate parasites (suspected)
- Inactive spermatogenesis
- Multifocal adrenocortical cysts
- Hypophyseal cysts

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-03
PATHOLOGY NO.: P2463-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested south of Hendrickson Island in Kugmallit Bay on 2015-07-10. It was from a group of six white and grey whales that included one newborn. This group appeared to be healthy and was travelling prior to the hunt. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Mild bronchopneumonia (parasitic implication suspected)**
2. **Splenic hemangioma**
3. **Molt**
4. **Inactive spermatogenesis**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed thin based on available pictures and morphometric measurements; he had the lowest body mass: total length ratio recorded (1.57), the smallest ½ maximal girth (90.2 cm) and dorsal epaxial musculature appears concave.

The lung lesions observed in this individual were mild and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal. The predominant mononuclear cells in conjunction with some eosinophils may indicate a chronic parasitic infection. Lungworms, mainly *Halocercus monoceris*, *H. taurica* and *Stenurus arctomarinus*, are the most frequent cause of parasitic pneumonia in Saint-Lawrence beluga whales (Lair 2016).

There is no report of hemangioma in beluga whales. This microscopic lesion is benign in appearance and did not impact the health of this animal.

The histology of the skin lesions observed in this animal are consistent with a normal molt process. Similar changes were noted in the spring migration as well as in the summer estuarine passage of Hudson Bay beluga whales (St. Aubin 1990).

The scarce presence of spermatozooids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References:

- Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. *Veterinary Pathology*. 2016 Jan;53(1):22-36.
- O'Brien JK, Steinman KJ, Schmitt T, Robeck TR. Semen collection, characterisation and artificial insemination in the beluga (*Delphinapterus leucas*) using liquid-stored spermatozoa. *Reproduction, Fertility and Development*. 2008 Oct 6;20(7):770-83.
- St Aubin DJ, Smith TG, Geraci JR. Seasonal epidermal molt in beluga whales, *Delphinapterus leucas*. *Canadian Journal of Zoology*. 1990 Feb 1;68(2):359-67.
- Stewart BE, Stewart RE. *Delphinapterus leucas*. *Mammalian Species*. 1989; (336): 1-8.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture on Hendrickson Island, 2015-07-11.

Sex: Male

Age: 20 years old

Weight: 595 kg

Body score (subjective): poor

Length: 379.8 cm

Carcass condition score: 2

External examination

An adult male, white in coloration with slightly curved flippers is examined. The harpoon is located just caudally and to the right of the dorsal crest. A single gunshot seemingly oriented caudo-ventrally is noted approximately 15 cm caudally to the blowhole. Multifocal, slightly depressed circular lesions with an irregular surface were distributed over the skin. Approximately 12 of those lesions were seen and their size usually ranged between 1 and 2 cm in diameter except for a larger one measuring approximately 3x5 cm.

Internal examination

Stomach and intestines: No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions: shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, lungs, heart, liver, lymph nodes (tracheobronchic, mesenteric), urinary system (bladder, kidney), reproductive tract (testes and epididymis), spleen.

HISTOPATHOLOGY

Skin (A): Normal skin is roughly 7 mm in thickness with a homogenous parakeratotic layer of 500 µm in thickness. Two abnormal skin sections are evaluated. Compared with the normal section, the parakeratotic layer is thicker, with small irregular projections of the most superficial cell layers. Several irregular cavities sometimes filled with an amorphous, acidophilic substance are noted within this irregular epithelium. Cells of this region vary in morphology and are variably vacuolated. In several cells, compact acidophilic structures are noted within the cytoplasm.

Spleen (C): A focal nodule of 2 mm in diameter, partially encapsulated is noted. Focal proliferative lesions are characterised by formation of papilliform projections lines composed of one layer of flat epithelium. These endothelial cells often contain multiple, acidophilic structures of a variable size within their cytoplasm and are variably vacuolated. Cavities formed by the projection lines contain blood.

Reproductive tract (C, E): No sperm is stored in the epididymis (E), and there is minimal spermatogenesis within the testis (C).

Lungs (G-L): Changes are similar on the different lung sections observed. Multifocally, the alveolar septum is thickened, in association with an infiltration of mononuclear and granulocytic (predominantly eosinophils) inflammatory cells. Histiocytes and eosinophils are also occasionally seen within the alveolar lumen. These lesions are usually mild and multifocal (G, H, I), but moderate and focally extensive reactions are also noted and may involve the bronchioles and intra-alveolar hemorrhages (H, I-p, K).

No significant lesions on the examined lesions from: heart (B), bladder (B), liver (C), Kidneys (D), muscle (D), pancreas (E), lymph node (E, F, N), thyroid (F), central nervous system (M/ N), adrenals (O to V)

Histomorphologic diagnostics

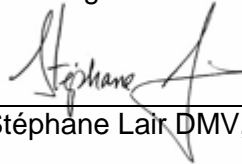
- Stratum externum degeneration
- Focal splenic hemangioma
- Inactive spermatogenesis
- Mild, multifocal eosinophilic bronchopneumonia

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-04
PATHOLOGY NO.: P2468-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested off of the southwest point of Hendrickson Island in Kugmallit Bay on 2015-07-11. It was from a group of approximately six white whales. The group appeared to be healthy and was travelling prior to hunt. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Stenurus* sp., *Halocercus* sp. suspected)**
2. **Renal nematodiasis (*Crassicauda giliakiana*)**
3. **Pituitary cysts**
4. **Adrenocortical cyst**
5. **Inactive spermatogenesis**
6. **Gastric nematodiasis**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed good based on available pictures and morphometric measurements; He was the third biggest whale in term of body mass (idem for body mass: length ratio – 2.28) and had the most important epaxial muscle weight recorded. He was also the longest whale measured and one of the largest ones. On available pictures, the skin surface cranial to the dorsal crest almost appears straight and the lumbar dorsal epaxial musculature appears well developed.

The size of the degraded structure observed in one section suggest that these lesions are associated of an infection with a pseudaliid nematode of the genus *Halocercus* (*H. monoceris* has been reported in monodontidae). In addition, although no nematode has been observed in the focal subpleural abscesses, these lesions are also described in chronic *Halocercus* sp. infections. The nematodes observed in the larger airways of this individual have a morphology that correspond to the pseudaliid nematode *Stenurus* sp. Despite these observations, the lung lesions in this individual were mild and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal (Lair 2016, Measures 2018).

The location and morphology of the encapsulated renal nematode is typical of the genus *Crassicauda* sp, a spirurid nematode found in mysticetes and odontocetes. *Crassicauda giliakiana* is generally site specific and associated to the urogenital system (Measures 2018). Although significant lesions can sometimes be associated with this parasite, in this animal the small proportion of renal tissue affected did not negatively impact its health.

Pituitary cysts containing colloid were a prominent feature in most cetaceans examined in a study of pathological features of odontocete cetaceans (Cowan 2008) and this observation is not considered to be significative.

Adrenocortical cysts have been described in Saint-Lawrence beluga whales and are thought to be associated with normal aging process, although they have also been suggested to be associated with the impact of stress or adrenocorticolitic xenobiotics (Lair 1997).

The scarce presence of spermatozoids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

- Cowan DF, Haubold EM, Tajima Y. Histological, immunohistochemical and pathological features of the pituitary gland of odontocete cetaceans from the western Gulf of Mexico. *Journal of Comparative Pathology*. 2008 Aug 1;139(2-3):67-80.
- Lair S, Beland P, De Guise S, Martineau D. Adrenal hyperplastic and degenerative changes in beluga whales. *Journal of Wildlife Diseases*. 1997 Jul;33(3):430-7.
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- Measures LN. Helminths and parasitic arthropods. In: Gulland FM, Dierauf LA, Whitman KL, editors. *CRC Handbook of Marine Mammal Medicine*. CRC Press; 2018. 471-497.
- O'Brien JK, Steinman KJ, Schmitt T, Robeck TR. Semen collection, characterisation and artificial insemination in the beluga (*Delphinapterus leucas*) using liquid-stored spermatozoa. *Reproduction, Fertility and Development*. 2008 Oct 6;20(7):770-83.
- Stewart BE, Stewart RE. *Delphinapterus leucas*. *Mammalian Species*. 1989; (336): 1-8.

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture on Hendrickson Island, 2015-07-11.

Sex: Male

Age: 32 years old

Weight: 1018 kg

Body score (subjective): good

Length: 446 cm

Carcass's condition score: 2

External examination

An adult male of a white coloration with moderately curved, black tipped flippers is examined. The whale has been harpooned caudally and to the right of the dorsal crest.

Internal examination

Lungs: The right lung is irregular in color; there are strains of darker, slightly collapsed parenchyma. Multiple hard nodules ranging from 3 to 7 mm in diameter are distributed multi-focally thorough the lung. Their center is sometimes necrotic. In some small airways, multiple black and white nematodes measuring approximately 1 x 60 mm are noted. No gross lesions or nematodes were noted in the left lung.

Kidneys: A white cystic structure of 1,5x 3 cm is noted on the surface of the right kidney. In the adjoining ureter, an approximately 20 x 0,5 cm yellow nematode is noted. On about 5 cm of its length, the nematode harbours a spiralled shape.

Stomach and intestines: A small quantity of nematodes is noted in the stomach's second compartment. No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions; shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, heart, liver, lymph nodes (tracheobronchic, mesenteric), bladder, intestines, reproductive tract (testes and epididymis), spleen.

HISTOPATHOLOGY

Reproductive tract (D, E): No sperm is stored in the epididymis (E), and there is minimal spermatogenesis within the testis (D).

Kidney (E, F): Portions of a thick fibrous capsule are noted (F). Damaged sections of a Thick, undulated cuticle are noted on the side of the section.

Lymph node (D): Focal mild accumulations of eosinophils are associated with radiated proteinaceous material. Occasionally, accumulation of giant cells phagocytising degenerated eosinophils and proteinaceous material are seen.

Lungs (G-L, M): All sections of the cranial and middle right lung have fully distended alveoli. Lesions in general are really mild, focal and generally consist of interstitial infiltration of mononuclear cells and rarely, eosinophils. The lumen of a single bronchiolo-alveolar junction of the right lung is filled with granulocytic cells (mainly eosinophils) and macrophages, including a giant multinucleated cell (G-p). Areas of fibroplasia sometimes associated with mononuclear (lymphoplasmacytic) infiltration are also noted, notably in the left lung (J-s, K-p). In these regions, the alveolar architecture is disrupted, including the integrity of the capillaries lining the alveolar walls.

In the left lung, a focal area of fibrosis and mononuclear cell infiltration with some eosinophils surrounds two small tubular areas of granulous acidophilic material including some giant multinucleated cells (L-m).

An additional section with one of the gross lesions described is examined (M). A 0.5 to 1 cm in diameter nodule distorts the pleural surface of the lung and compresses adjacent alveolar parenchyma. Massive infiltration of mononuclear cells (macrophages), including giant multinucleated cells, as well as eosinophils are intertwined with disorganised fibrous strands. Type II pneumocyte hyperplasia is seen in the remaining alveoli at the margin of the lesion. The center of the nodule is composed of smaller nodules, surrounded by varying thicknesses of fibrous and macrophages lamellar deposition. In their center, there are granular, eosinophilic and basophilic granular material, degenerate granular cells and neutrophils.

Hypophysis (M): Multiple enlarged cortical follicles (<0.1 mm) are noted in two different clusters, in proximity to small cysts (2) of 0.3-0.5 mm in diameter, lined by a cuboidal epithelium and filled with a pale eosinophilic liquid.

Adrenals (Q to W): A single, 0.52 mm cortical cyst is noted (U).

No significant lesions on the examined sections from: Skin (A), bladder (A), heart (B), thyroid (B), muscle (B), pancreas (C), spleen (C), liver (C) cerebrum (N), cerebellum (P), medulla oblongata (O),

Histomorphologic diagnostics:

- Chronic, mild, multifocal interstitial parasitic bronchopneumonia and multifocal purulo-granulomatous pneumonia
- Focal renal encapsulated nematodiasis (*Crassicauda giliakiana*)
- Hypophyseal cysts
- Focal adrenocortical cyst
- Inactive spermatogenesis

Resident:

Émilie L. Couture, DMV, IPSAV, DES

Pathologist:

Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-05
PATHOLOGY NO.: P2469-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested south of Hendrickson Island in Kugmallit Bay on 2015-07-11. It was travelling alone prior to the hunt. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous granulomatous pneumonia (*Stenurus* sp.)**
2. **Inactive spermatogenesis**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This young male's body condition was deemed thin (poor) based on available pictures and morphometric measurements; he had one of the lowest body mass: total length ratio recorded (1.60), and dorsal epaxial musculature appears concave. A poor body condition is not unexpected in a wild, migrating animal.

The morphology of the parasite observed in the airways as well as associated lesions are typical of an infection with a pseudaliid nematode of the genus *Stenurus* (*S. arctomarinus* has been reported in monodontidae). Pathogeny associated with these lungworms is typically associated to airway occlusion due to the physical presence of parasite within airways. In this case, the parenchymal localisation of the parasite was associated with a marked granulomatous reaction. Despite these observations, the lung lesions in this individual were mild and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal (Lair 2016, Measures 2018).

The scarce presence of spermatozooids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

- Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. *Veterinary Pathology*. 2016 Jan;53(1):22-36.
- Measures LN. Helminths and parasitic arthropods. In: Gulland FM, Dierauf LA, Whitman KL, editors. *CRC Handbook of Marine Mammal Medicine*. CRC Press; 2018. 471-497.
- O'Brien JK, Steinman KJ, Schmitt T, Robeck TR. Semen collection, characterisation and artificial insemination in the beluga (*Delphinapterus leucas*) using liquid-stored spermatozoa. *Reproduction, Fertility and Development*. 2008 Oct 6;20(7):770-83.
- Stewart BE, Stewart RE. *Delphinapterus leucas*. *Mammalian Species*. 1989; (336): 1-8.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair, Dr. Émilie L. Couture and Sonia Ostertag, PhD on Hendrickson Island, 2015-07-11.

Sex: Male

Age:

Weight: 574 kg

Body score (subjective): Poor

Length: 353.1 cm

Carcass condition score: 2

External examination

An adult male of a white coloration with moderately curved, black tipped flippers is examined. The whale has been harpooned caudally to the dorsal crest. Two gunshot wounds are located on the right thoracic wall.

Internal examination

Lungs: In both lungs, some small occasional black and white nematodes measuring approximately 1 x 60 mm are noted in the small airways.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions: shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, heart, liver, lymph nodes (tracheobronchic, mesenteric), urinary system (bladder, kidneys), reproductive tract (testes and epididymis), spleen.

HISTOPATHOLOGY

Reproductive tract (D, N): No sperm is stored in the epididymis (D), and there is no spermatogenesis within the testis (N).

Lungs (E-J+K): There are occasional focal, mild to moderate thickening of the alveolar, peribronchiolar and vascular septum by mononuclear (histiocytes, lymphocytes) inflammatory cell infiltration, sometimes associated with the presence of acidophilic amorphous material and granulocytes within surrounding alveolar lumens (E, F, G). In some sections part of a nematode, approximately 0.8 mm in diameter and with a thick, pigmented cuticle and digestive tract, are noted (F, H, I). The nematode is surrounded by a thin fibrous capsule with foci of mineralization and multiple layers of mononuclear and granulocytic cells between fibrocytes. The parenchymal inflammatory infiltration is severe in this region, but only a pale amorphous proteinaceous material may be found within some surrounding alveolar lumen. Discrete foci of mineralisation are also noted within parenchymal inflammatory infiltrations. Two granulomas with a suppurative necrotic center are also noted (I) on a section. A focal mild perivascular eosinophilic infiltration is also noted (J). While described lesions have been noted on 50% of a section, the proportion of affected lung is generally lower and ranges between 0 and 15%.

No significant lesions on the examined lesions from: skin (A), bladder (A), heart (B), muscle (B), spleen (C), liver (C), thyroid (C), kidneys (D), pituitary (K), central nervous system (L, M), lymph node (N), adrenals (O to T)

Histomorphologic diagnostics

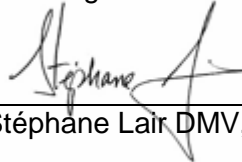
- Multifocal, mild verminous granulomatous pneumonia (*Stenurus* sp.).
- Inactive spermatogenesis

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-06
PATHOLOGY NO.: P2470-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult female beluga whale was harvested east of Hendrickson Island in Kugmallit Bay on 2015-07-11. It was travelling alone prior to the hunt. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Chronic pneumonia (parasitic origin likely)**
2. **Muscle sarcocystosis**
3. **Gestation – first trimester**
4. **Active lactation**
5. **Ovarian cysts**
6. **Pituitary cysts**
7. **Adrenal cysts and cortical nodular hyperplasia**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed fair based on available pictures and morphometric measurements; She was the only female weighed and has a body mass: total length ratio of 1.75. On available pictures, the lumbar dorsal epaxial musculature is adequate, forming a straight line from midline to flanks.

The size of the foetus indicates that this old female was bred earlier in the same year. The signification of the ovarian cysts noted in this individual is unknown. They were likely not associated with significant hormone production as this did not preclude this female from being pregnant. An active lactation might indicate that she had a suckling veal born in the previous summer or that she was participating in allonursing (Leung 2010).

The species of sarcocysts found in the muscles is unknown, although we are looking to pursue additional diagnostic. Only two species are known to infect humans through the consumption of meat. They are found in beef and pork and may cause digestive signs (nausea, vomiting enteritis), although most infected persons will show mild or no signs at all. Freezing or cooking of the meat is recommended, as it has been shown to kill sarcocysts (Fayer 2015).

The lung lesions observed in this individual were mild and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal. The predominant mononuclear cells in conjunction with some eosinophils, as well as areas of fibroplasia may indicate a chronic parasitic infection. Lungworms, mainly *Halocercus monoceris*, *H. taurica* and *Stenurus arctomarinus*, are the most frequent cause of parasitic pneumonia in St. Lawrence beluga whales (Lair 2016).

Pituitary cysts containing colloid were a prominent feature in most cetaceans examined in a study of pathological features of odontocete cetaceans (Cowan 2008) and this observation is not considered to be significant.

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes. Likewise, adrenocortical nodular hyperplasia has been reported in adult (>15 years old) beluga whales from the St. Lawrence estuary and is stipulated to be part of a normal aging process or to alternatively constitute a regenerative response to degenerative lesions (Lair, 1997).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

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DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture on Hendrickson Island, 2015-07-11.

Sex: Female

Age: 45

Weight: 622 kg

Body score (subjective): Good

Length: 350.6 cm

Carcass's condition score: 2

External examination

An adult female of a white coloration is examined. The whale has been harpooned caudally and to the right of the dorsal crest. Gunshot wounds (4) are noted on the dorsal left thoracic wall and on the left side of the neck. Multifocal, slightly depressed circular lesions with an irregular surface were distributed over the skin. Approximately 20 of those lesions were seen and their size ranged between 1 and 2 cm in diameter. A focal area of grey and irregular tissue is noted on the anterior junction of the left flipper.

Internal examination

Stomach and intestines: No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

Uterus: A male foetus of approximately 20 cm in length is located in a uterine horn. No gross abnormalities are noted on the foetus.

Mammary gland: An active lactation is noted.

Ovaries: Four cysts ranging from 1 to 4 cm in diameter are noted on the right ovary. On the left ovary, two corpora lutea are noted. One is 2 cm in diameter while the other one is 4.5 cm in diameter.

The following structures were without noticeable gross lesions: shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, heart, liver, lymph nodes (tracheobronchic, mesenteric), urinary system (bladder, kidneys), spleen.

HISTOPATHOLOGY

Uterus (A): The whole thickness of the endometrium is not evaluated.

Muscle (B): Cyst-like structures are noted in a few muscular fibers and contain numerous small and elongated basophil structures (Sarcocysts).

Mammary gland (C): Epithelial cells are gorged with fat droplets and proteinaceous material. Proteinaceous liquid is noted within intralobular canals.

Lungs (E to J): Mild, multifocal and generally discrete interstitial mononuclear cell infiltration, sometimes associated with eosinophil and alveolar macrophages within airways. More extensive interstitial infiltrations are associated with a greater proportion of eosinophils, as well as with the additional presence of erythrocytes within luminal secretions. Eosinophils may also be seen in arteriolar walls (E-p). Areas of fibroplasia, associated or not with active inflammatory cell infiltration are noted (e.g. G-p, H-m)

Ovary (K to Q): Numerous follicles at different development stages are noted, up to antral follicles. A large corpus luteum is noted (O). At least one corpus albicans, with partial mineralization is noted (Q). Multiple cystic structures, lined by multiple loose layers of conjunctive tissue and a single cuboidal epithelium are noted in the right ovary (K, L, P).

Adrenal (U to Z): Approximately 10 medullar and cortical cysts of 0.6 to 1.3 mm are noted in both adrenal glands (U,V, X, Y, Z). Multiple coalescing small cysts are also noted and are displaying a characteristic spiderweb pattern, for a total of up to 2.5 mm in diameter (X, Z) as well as a large cortical hyperplastic nodule of 10 mm in diameter on the right adrenal (V). Smaller clusters of basophilic cells (Y) are also noted

Pituitary (R): Cystic structures, 0.1 to 0.3-0.5 mm in diameter and lined with a cuboidal epithelium, are noted in the adenohypophysis (pars distalis). Multiple large follicles (<0.1 mm) are also noted and are sometimes aggregated together.

No significant lesions on the examined lesions from: Skin (A), heart (B), liver (C), thyroid (C), spleen (D), kidney (D), cerebrum (S,T), cerebellum (R, T)

Histomorphologic diagnostics

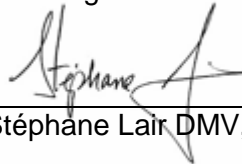
- Multifocal eosinophilic bronchopneumonia with areas of interstitial fibroplasia
- Multifocal muscular sarcocystosis
- Diffusely active lactocytes
- Right ovarian cysts
- Active left ovarian activity, as well one corpus albicans and one corpus luteus (pregnancy)
- Multifocal adrenocortical cysts and focal adrenocortical hyperplasia.
- Multifocal pituitary cysts

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-08
PATHOLOGY NO.: P2471-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested in Kugmallit Bay on 2015-07-11. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Chronic parasitic bronchopneumonia (*Stenurus* sp., possibly *Halocercus* sp.)**
2. **Molt**
3. **Inactive spermatogenesis**
4. **Nodular renal fibrosis (*Crassicauda* sp suspected)**
5. **Renal medullar and epididymal epithelium multifocal mineralisation**
6. **Mesenterium nodular fibrosis**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed fair based on available pictures and morphometric measurements; He has a relatively small male with body mass: total length ratio of 1.76. On available pictures, the lumbar dorsal epaxial musculature is adequate, forming a straight line from midline to flanks.

Most lung lesions observed in this individual were generally mild and of a chronic nature, while active inflammatory processes still affected larger areas in some sections (max: 25% of a section). The nematodes observed in the larger airways of this individual correspond to pseudaliid nematodes of the genus *Stenurus* sp. Although their role in bronchopneumonia lesions cannot be excluded, observations also correspond to typical lesions associated with pseudaliid nematodes of the genus *Halocercus* that may not have been present on examined sections. Despite these observations, the lung lesions in this

individual were mild and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal (Lair 2016, Measures 2018).

The histology of the skin lesions observed in this animal are consistent with a normal molt process. Similar changes were noted in the spring migration as well as in the summer estuarine passage of Hudson Bay beluga whales (St. Aubin 1990).

The scarce presence of spermatozooids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

Although no etiology was found in association with the fibrous nodule found in the kidney, the most common reason for a similar lesion would be an encapsulated renal nematode of the genus *Crassicauda* sp, a spirurid nematode found in mysticetes and odontocetes (Measures 2018). Although significant lesions can sometimes be associated with this parasite, in this animal it is possible that the fibrous capsule is only an indication of the prior presence of a parasite, and the small proportion of renal tissue affected did not negatively impact its health.

The brightly staining structures with the PAS stain in muscular fibers could potentially represent an artefact associated with the presence of glycogen. Although sarcocyst bradyzoites usually contain prominent amylopectin granules that stain brightly with PAS stain, this option appears less likely as degenerate parasitic cysts are described to be associated with some sort of inflammatory reaction (Cooper 2016, Verma 2017).

The renal medullar and epididymal multifocal mineralization foci are considered to be incidental finding. The association of the mesenterium fibrous nodule with similar structures observed in the mesenteric lymph nodes of some individuals is unknown. Please note that lesions deemed associated with the harvesting method are not described in this report.

References

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 - Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. *Veterinary Pathology*. 2016 Jan;53(1):22-36.
 - Measures LN. Helminths and parasitic arthropods. In: Gulland FM, Dierauf LA, Whitman KL, editors. *CRC Handbook of Marine Mammal Medicine*. CRC Press; 2018. 471-497.
 - O'Brien JK, Steinman KJ, Schmitt T, Robeck TR. Semen collection, characterisation and artificial insemination in the beluga (*Delphinapterus leucas*) using liquid-stored spermatozoa. *Reproduction, Fertility and Development*. 2008 Oct 6;20(7):770-83.
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 - Stewart BE, Stewart RE. *Delphinapterus leucas*. *Mammalian Species*. 1989; (336): 1-8.
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DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. on Hendrickson Island, 2015-07-11.

Sex: Male

Age: 22

Weight: 683 kg

Body score (subjective): fair

Length: 388.7 cm

Carcass condition score: 2

External examination

An adult male of a white coloration with markedly curled flippers was examined. The whale was harpooned caudally to the dorsal crest and a single gunshot wound is noted on the left side of the neck. Multifocal, slightly depressed circular lesions were distributed over the skin. Approximately 12 of those lesions were seen and their size ranged between 1 and 2 cm in diameter.

Internal examination

Lungs: Occasional black and white nematodes measuring approximately 1 x 60 mm are noted in the small airways of the right lung.

Kidney: A white nodule of 2 cm in diameter and of a firm, bony consistency is found in a kidney.

Mesenterium: A white nodule of 2 cm in diameter and of a firm, bony consistency is found embedded in the mesenterium.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions; shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, heart, liver, lymph nodes (tracheobronchic, mesenteric), bladder, reproductive tract (testes and epididymis), spleen.

HISTOPATHOLOGY

Skin and blubber (A): The distinction between the stratum externum and stratum intermedium is not well defined. Irregular, flattened layers of keratin debris and degenerate squamous cells are observed at the most superficial portion of the stratum externum. Longitudinal clusters of polyhedral cells are seen between this and normal underlying stratum externum.

Muscle (B, D): A muscular fiber contains numerous streaks and clusters of finely granular material not picking the coloration. On PAS stain, similar structures are filled with granulous material with a bright purple coloration, indicating these structures contain glycogen.

Reproductive tract (D, F): No sperm is stored in the epididymis (D), and there is no spermatogenesis within the testis (F). Multifocal foci of mineralisation are seen within the epididymal epithelium. There is mild to moderate interstitial fibrosis in the testis.

Kidneys (E): There is multifocal mineralization within medullar collecting tubules. On one section (E1), there is a 0.5x 1cm in diameter thick fibrous nodule, with fibroblasts mostly present in its periphery.

Lungs (G to L): There is mild, mononuclear inflammatory cells interstitial infiltration surrounding bronchioles, arterioles. Sometimes associated with multinucleated cells, and rarely with eosinophils. In some sections, infiltrations of mononuclear cells are patchy and associated with increased collagen deposition, focally disrupting the parenchymal architecture (G-m). Occasionally, cellular infiltrations are

associated with the presence of intraluminal (bronchiole, alveolar) macrophages and some eosinophils. In sections where damages are more locally extensive, the luminal and interstitial proportion of eosinophils is more important and small hemorrhages may be seen (H-p, J-s).

No significant lesions on the examined lesions from: Bladder (A), heart (B), liver (C), spleen (C), pancreas (C), thyroid (D), lymph nodes (F) central nervous system (M, N, O), hypophysis (O), adrenals (P to U).

Histomorphologic diagnostics

- Irregularity and degeneration of the most superficial portion of the stratum externum
- Inactive spermatogenesis
- Focal renal fibrosis
- Multisystemic multifocal mineralisation (renal medulla, epididymis)
- Chronic mild, multifocal eosinophilic (parasitic) bronchopneumonia and fibroplasia

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-12
PATHOLOGY NO.: P2472-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested southwest of Hendrickson Island in Kugmallit Bay. It was travelling alone prior to the hunt. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Halocercus* sp. likely)**
2. **Renal nematodiasis (*Crassicauda* sp)**
3. **Lymphonodular trematodiasis (probable)**
4. **Molt**
5. **Inactive spermatogenesis**
6. **Adrenocortical cyst**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed fair based morphometric measurements and pictures. Body mass: total length ratio of 1.94. On available pictures, the lumbar dorsal epaxial musculature is adequate, forming a straight line from midline to flanks.

Most lung lesions observed in this individual were generally mild and of a chronic nature. Mineralized parasitic remnants likely correspond to a pseudaliid nematode of the genus *Halocercus*. Despite these observations, the lung lesions in this individual were mild and did not affect its health considering the

absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal (Lair 2016, Measures 2018).

The location and morphology of the encapsulated renal nematode is typical of the genus *Crassicauda* sp, a spirurid nematode found in mysticetes and odontocetes. *Crassicauda giliakiana* is generally site specific and associated to the urogenital system (Measures 2018). Although significant lesions can sometimes be associated with this parasite, in this animal the small proportion of renal tissue affected did not negatively impact its health.

Although it was not possible to confirm an etiology in association with the lymph node nodules, trematode eggs have been observed in similar lesions of other beluga whales of this population. In Hector's dolphins (*Cephalorhynchus hectori*), an adult trematode had a morphology corresponding to *Campula* spp was noted to be encapsulated in the mesenteric lymph nodes, along with similarly encapsulated aggregates of trematode eggs associated with a strong macrophagic and fibrosing reaction (Hutton 1987). In general, digenean trematode of the genus *Campula* are associated with the liver, bile and pancreatic apparatus in odontocetes. They may cause variable level of lesions and their life cycle is poorly known (Measures 2018). In this case, the nodules were unlikely to have a negative health effect.

The histology of the skin lesions observed in this animal are consistent with a normal molt process. Similar changes were noted in the spring migration as well as in the summer estuarine passage of Hudson Bay beluga whales (St. Aubin 1990).

The scarce presence of spermatozooids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes (Lair, 1997).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

- Hutton J, Blair D, Slooten E, Dawson SM. Case-studies of fluke-induced lesions in mesenteric lymph-nodes of Hector's dolphins *Cephalorhynchus hectori*. Diseases of Aquatic Organisms. 1987 Apr 15;2(2):83-6.
- Lair S, Beland P, De Guise S, Martineau D. Adrenal hyperplastic and degenerative changes in beluga whales. Journal of Wildlife Diseases. 1997 Jul;33(3):430-7.
- Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. Veterinary Pathology. 2016 Jan;53(1):22-36.
- Measures LN. Helminths and parasitic arthropods. In: Gulland FM, Dierauf LA, Whitman KL, editors. CRC Handbook of Marine Mammal Medicine. CRC Press; 2018. 471-497.
- O'Brien JK, Steinman KJ, Schmitt T, Robeck TR. Semen collection, characterisation and artificial insemination in the beluga (*Delphinapterus leucas*) using liquid-stored spermatozoa. Reproduction, Fertility and Development. 2008 Oct 6;20(7):770-83.
- St Aubin DJ, Smith TG, Geraci JR. Seasonal epidermal molt in beluga whales, *Delphinapterus leucas*. Canadian Journal of Zoology. 1990 Feb 1;68(2):359-67.

- Stewart BE, Stewart RE. *Delphinapterus leucas*. Mammalian Species. 1989; (336): 1-8.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture on Hendrickson Island, 2015-07-13.

Sex: Male

Age: 20

Weight: 750 kg

Body score (subjective): Fair

Length: 386.2 cm

Carcass condition score: 2

External examination

An adult male of a white coloration is examined. The harpoon was located caudally and to the left of the dorsal crest. A gunshot is noted on the left side of the neck. Multifocal, slightly depressed circular lesions with an irregular surface were distributed over the skin. Approximately 30-40 of those lesions were seen and their size ranged between 1 and 2 cm in diameter.

Internal examination

Lymph nodes: All mesenteric lymph nodes are moderately enlarged. Approximately half of them contain numerous white nodules. These nodules are of a bony consistency, have a necrotic center and vary from 3-7 mm in diameter.

Kidneys: Three cysts are noted in the left kidney.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions

shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, heart, liver, tracheobronchic lymph nodes, bladder, reproductive tract (testes and epididymis), spleen.

HISTOPATHOLOGY

Skin (A): A thick layer of large polyhedral and acidophilic cells with a pyknotic nucleus and an irregular arrangement is noted on both sections examined (degenerate cell layer). On one of the sections, there is a crateriform shed of the most superficial portion of this layer. Underneath this layer (DCL), a new stratum externum is in formation (gradual flattening of epithelial cells)

Kidney (G, H, I): Several sections of nematode, 5 mm in diameter, with a thick pigmented cuticle are noted (H, I). They are encapsulated in a thick fibrous capsule and are surrounded by amorph proteinaceous material. Scarce eosinophils may be noted within the capsule. A ureter is visible, adjacent to one of the fibrous capsule.

Reproductive tract (D, E): No sperm is stored in the epididymis (D), and there is no spermatogenesis within the testis (E). Multifocal foci of mineralisation are seen within the epididymal epithelium.

Lymph node (D, E, F, V): Numerous active germinal centers are noted in the paracortical area. Multifocal, thick fibrous cysts surrounded by a mild granulocytic and macrophages infiltration are noted. Fibrous capsule may sometimes present mineralisation. The central portion of the cysts consists of a necrotic center composed of degenerated inflammatory cells and mineralisation foci. The surrounding medullary sinuses are distended by oedematous fluid in which eosinophils may occasionally be seen. In some areas, focal accumulations of eosinophils are centered in proteinaceous, radiated material. A focal degenerated structure suggests parallel muscular fibres. No alchoolo-acido resistant bacilli, bacteria or fungus are noted on Zielh-Neelsen, Gram (Brown-Hopps) and PAS stains, respectively. Von Kossa stain reveals that the mineralization centers contain calcium salts.

Lungs (K to L): In some sections, multiple airways (alveoli, bronchioles) contain eosinophils and alveolar macrophages in addition to proteinaceous material (K-s). There is mild multifocal mononuclear cell infiltration, sometimes associated with fibroblast and fibrous deposition. A mineralized center with granular proteinaceous material that seem to take a circular, 0.1mm in diameter form is seen (N-p). In one section, fine strands of fibrous tissue create and hypersegmentation of the alveoli and bronchioles (L-p) Adrenals (T to Y): A single cystic formation of 0.52 mm is noted and is formed by 3 adjacent cysts (Y).

No significant lesions on the examined lesions from: Bladder (B), duodenum (B), pancreas (B), muscle (C), heart (C), thyroid (J), spleen (J), liver (J), cerebrum (R), cerebellum (S), medulla oblongata (Q), pituitary gland (Q)

Histomorphologic diagnostics

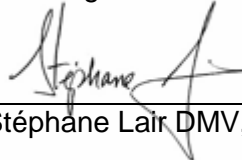
- Epidermal superficial degenerate cell layer shedding
- Fibro-nodular renal nematodiasis
- Inactive spermatogenesis
- Multifocal fibro-necrotic lymphonodular nodules.
- Mild multifocal fibrosing interstitial pneumonia with probable parasitic remnants and moderate eosinophilic broncho-alveolitis.
- Focal adrenocortical cyst

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-13
PATHOLOGY NO.: P2473-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested southeast of Hendrickson Island in Kugmallit Bay. It was swimming around with a group of six white and grey whales. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Chronic cheilitis, associated with a penetrating foreign body**
2. **Molt**
3. **Verminous bronchopneumonia (probable)**
4. **Multifocal discrete renal medullary mineralization**
5. **Inactive spermatogenesis**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed fair based morphometric measurements. Body mass: total length ratio of 1.95. No pictures were available for a visual assessment.

The inflammatory reaction noted in one skin section, appears to be associated with the presence of foreign objects, some of which are below the epidermal layer. This material might be the origin of the suppurative and histiocytic inflammatory reaction and although the location of sampling has not been noted, could be associated with the lesion described in the corner of the mouth. The histology of the other skin sections observed in this animal are consistent with a normal molt process. Similar changes were

noted in the spring migration as well as in the summer estuarine passage of Hudson Bay beluga whales (St. Aubin 1990).

Most lung lesions observed in this individual were generally mild and of a chronic nature, while sections with a larger surface area involved generally had a more active inflammatory process. Despite these observations, the lung lesions in this individual were mild and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal. Although no etiology could be confirmed on the examined sections, lungworms, mainly *Halocercus monoceris*, *H. taurica* and *Stenurus arctomarinus*, are the most frequent cause of parasitic pneumonia in Saint-Lawrence beluga whales (Lair 2016, Measures 2018).

The scarce presence of spermatozoids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989). The multifocal discrete renal medullary mineralization is considered incidental.

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

- Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. *Veterinary Pathology*. 2016 Jan;53(1):22-36.
- Measures LN. Helminths and parasitic arthropods. In: Gulland FM, Dierauf LA, Whitman KL, editors. *CRC Handbook of Marine Mammal Medicine*. CRC Press; 2018. 471-497.
- O'Brien JK, Steinman KJ, Schmitt T, Robeck TR. Semen collection, characterisation and artificial insemination in the beluga (*Delphinapterus leucas*) using liquid-stored spermatozoa. *Reproduction, Fertility and Development*. 2008 Oct 6;20(7):770-83.
- St Aubin DJ, Smith TG, Geraci JR. Seasonal epidermal molt in beluga whales, *Delphinapterus leucas*. *Canadian Journal of Zoology*. 1990 Feb 1;68(2):359-67.
- Stewart BE, Stewart RE. *Delphinapterus leucas*. *Mammalian Species*. 1989; (336): 1-8.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture, Hendrickson Island, 2015-07-13.

Sex: Male

Age: 22

Weight: 782 kg

Body score (subjective): Good

Length: 401.4 cm

Carcass condition score: 2

External examination

An adult male of a white coloration is examined. Occasional crateriform circular depressions are found on the skin surface. A 15 cm in size lesion is noted on the right corner of the mouth.

Internal examination

Liver: a 5x1 cm white scar is noted on the marginal serosa of the liver.

Spleen: The spleen is twice its usual size.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions: shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, heart, liver, lymph nodes (tracheobronchic, mesenteric), urinary system (bladder, kidneys), reproductive tract (testes and epididymis), spleen.

HISTOPATHOLOGY

Skin and blubber (A): Three sections are examined. One is normal, with a thick stratum externum. On the second one, the previous stratum externum appears to have been recently shed as irregular cellular debris are seen on the surface and a new layer of flattened stratum intermedium is seen underneath. On the third examined section, there is multifocal to coalescent infiltration of mononuclear inflammatory cells within dermal papillae and superficial blubber, just under the stratum germinativum. Giant multinucleated cells are also present. A strand of polyhedral cells with anisocytosis and anisokaryosis, sometimes with two nucleuses is seen extending in the dermis in association with the mononuclear inflammatory cell aggregation. Aggregations of degenerated cells and neutrophils is also present in the stratum germinativum and within this latter cellular strand, as well as round structures, acidophilic to basophilic and with a nucleus of a varied shape. No bacteria is noted on Gram-Twort coloration but the round structures appear refractile and additional, rectangular, cork-like fragments are noted (foreign bodies). What appears to be an oblique section of a cylindrical, partially mineralized structure is seen at the interface between the dermis and the epidermis. The overlying epidermal layers are markedly disorganised and composed of swirls of variably swollen large polyhedral cells with occasional round acidophilic with a basophilic nucleus intracytoplasmic structure.

Reproductive tract (D, E): No sperm is stored in the epididymis (D), and there is no spermatogenesis within the testis (E). Multifocal foci of mineralisation are seen within the epididymal epithelium.

Kidneys (H): Multifocal punctate mineralization within blood vessels in the renal medulla.

Lungs (H to N): multifocal mild and discrete infiltration of mononuclear inflammatory cells. Lesions with a greater surface area are associated with a larger proportion of eosinophils in the interstitial tissue and within alveoli (with alveolar macrophages and some erythrocytes). Sometimes, more diffuse tracts of patchy mononuclear infiltration are associated with mild fibroplasia (N-s).

No significant lesions on the examined lesions from: bladder (B), duodenum (B), heart (C), muscle (C, G), lymph node (D, E), thyroid (F), liver (F), pancreas (G), spleen (G), cerebellum (O, P), pituitary (O), adrenals (Q to V)

Histomorphologic diagnostics

1. Focally extensive mononuclear and suppurative dermatitis associated with foreign bodies
2. Degenerate cell skin layer shedding
3. Mild multifocal eosinophilic bronchopneumonia and interstitial fibroplasia
4. Multifocal discrete renal medullary mineralization
5. Inactive spermatogenesis

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-14
PATHOLOGY NO.: P2474-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult female beluga whale was harvested southwest of Hendrickson Island in Kugmallit Bay on 2015-07-14. It was in a group of three white whales that appeared to be travelling prior to the hunt. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Post-reproductive female with active lactation**
2. **Verminous bronchopneumonia (*Halocercus* sp.)**
3. **Molt**
4. **Thyroid nodular adenomatous hyperplasia and cysts**
5. **Adrenocortical cysts**

Interpretation and comments:

None of the conditions noted in this individual was deemed to negatively affect its general condition. This animal's body condition was deemed thin based on available pictures and morphometric measurements; no body mass was obtained, but the epaxial muscle weight, blubber depths and ½ girths were within the lower recorded between different whales. In addition, dorsal epaxial musculature appear sunken on available pictures.

This older female was not pregnant and did not appear to be reproductively active considering the lack of ovarian follicles. However, the presence of corpus albicans and suggests past pregnancies. The

female might have been participating in allonursing, or less likely, lactating for an older calf of her own (Leung 2010).

Most lung lesions observed in this individual were generally mild and of a chronic nature. Whole and degenerate parasitic remnants in more prominent lesions as well as a mineralized granuloma likely correspond to a chronic-active pseudaliid nematode infection of the genus *Halocercus*. Despite these observations, the lung lesions in this individual were mild and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal (Lair 2016, Measures 2018).

The histology of the skin lesions observed in this animal are consistent with a normal molt process. Similar changes were noted in the spring migration as well as in the summer estuarine passage of Hudson Bay beluga whales. (St. Aubin 1990).

Follicular cysts and adenomatous hyperplasia have been described in both arctic (Hudson Bay) and St. Lawrence beluga populations. Cysts did not appear to be correlated with age and did not appear to have a clinical implication nor a clear suspected etiology, while the volume of adenomatous hyperplasia was positively correlated with age (Mikaelian 2003).

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes (Lair, 1997).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

- Lair S, Beland P, De Guise S, Martineau D. Adrenal hyperplastic and degenerative changes in beluga whales. *Journal of Wildlife Diseases*. 1997 Jul;33(3):430-7.
- Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. *Veterinary Pathology*. 2016 Jan;53(1):22-36.
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- Mikaelian I, Labelle P, Kopal M, De Guise S, Martineau D. Adenomatous hyperplasia of the thyroid gland in beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary and Hudson Bay, Quebec, Canada. *Veterinary Pathology*. 2003 Nov;40(6):698-703.
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DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture, Hendrickson Island, 2015-07-14.

Sex: Female

Age:

Weight: N/A

Body score (subjective): Fair

Length: 345.6 cm

Carcass condition score: 2

External examination

An adult female of a white coloration is examined. The whale has been harpooned dorso-laterally at the level of the anus, on the left side. Gunshots wounds (2) are noted dorsally to the neck and on the dorsal left portion of the thorax. Occasional target-like circular depressions of a yellow to light brown coloration and 1 to 2 cm in diameter are found on the skin surface.

Internal examination

Mammary gland: A small amount of milk is expressed upon compression of the gland.

Ovaries: No corpus luteum noted.

Uterus: The uterine horns are small and symmetrical. A mucus plug is noted on the cervix.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions; shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, heart, liver, lymph nodes (tracheobronchic, mesenteric), urinary system (bladder, kidneys), reproductive tract, spleen.

HISTOPATHOLOGY

Skin and blubber (A): Two sections are examined. On one, a thickened stratum externum is partially shed. On the other, the stratum externum is relatively thin and partially eroded, in association with the presence of granular material, possible microorganisms and vegetal material.

Muscle (B, F): Numerous muscle fibers are fragmented, and the sarcoplasm is replaced by a fine granular material (B).

Thyroid (C): There is a marked difference in thyroid follicle shape through the examined sections. While many appear almost empty, several larger follicles of 1 mm as well as cystic follicles of up to 5 mm in diameter are found, often with a distorted shape associated with invagination of the follicular wall. The epithelium lining follicles and cysts is cuboidal to cylindrical and appears hyperplastic. On one section, a 5 mm in diameter encapsulated region of irregular follicles is noted and compresses the adjacent parenchyma. Within this capsule, follicular cells appear enlarged when compared to the rest of the gland. Follicles present a papillary form and often contain clear vacuoles. Colloid may be seen between the follicles. Moderate anisokaryosis is present.

Mammary gland (D): Mostly inactive, some alveoli still contain a small amount of proteinaceous liquid.

Ovaries (H, I): both ovaries are quiescent. No follicles, at any stage of maturation are seen on either ovaries. Multifocal regions of fibrosis are noted within the parenchyma (corpus albicans)

Lungs (J to O): Multifocal, mild, discrete to locally extensive interstitial accumulation of mononuclear inflammatory cells (macrophages) and eosinophils. Affected surfaces may be larger and involve intraluminal (alveolar, bronchiolar) accumulation of alveolar macrophages, eosinophils and sometimes erythrocytes. Approximately 0.1 mm in diameter cylindrical proteinaceous debris or intact nematode sections may be seen within lesions and may be associated with multinucleated giant cells (L-p, M-m, N-m, O-s). Arteriolar infiltration of eosinophils is also noted (N-s). Focally (O-m), a fibrous granuloma with a mineralized center is noted. Cartilage mineralisation in terminal bronchi is noted (K).

Adrenal (R to W): Multiple (more than 15, marked) cystic formation ranging from 0.52 mm to 3 mm in diameter, formed by varying numbers of cysts of varying size separated by fine connective tissue (characteristic spider web appearance) and lined with a flattened epithelium. Surrounding cells sometimes present with vacuolisation. Multiple mineralization foci are noted in the periphery of some cysts or cystic formation (S, T, U).

No significant lesions on the examined lesions from: heart (B), bladder (B), lymph node (D), pancreas (E), spleen (F), liver (F), kidneys (G), central nervous system (P, Q)

Histomorphologic diagnostics

- Shedding and erosion of superficial stratum externum
- Thyroid nodular adenomatous hyperplasia and follicular cysts
- Multifocal, mild to moderate eosinophilic and granulomatous parasitic bronchopneumonia (*Halocercus* sp.)
- Quiescent ovaries with some corpus albicans.
- Multifocal adrenocortical cysts
- Minimal milk production

Resident:

Émilie L. Couture, DMV, IPSAV, DES

Pathologist:

Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-15
PATHOLOGY NO.: P2475-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult female beluga whale was harvested southwest of Hendrickson Island in Kugmallit Bay on 2015-07-16. It was in a large group of more than 20 whales that included some black and grey whales. The group was travelling towards the Mackenzie River prior to the hunt. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. Muscular sarcocystosis
2. Lymphonodular trematodiasis
3. Partial lactation
4. Gestation – first semester
5. Adrenocortical cysts
6. Mild bronchopneumonia (non significant)

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed fair based on limited available pictures and morphometric measurements. A total body mass is not available but epaxial muscular weight and blubber depths compared favorably with males of the same size.

The species of sarcocysts found in the muscles is unknown, although we are looking to pursue additional diagnostic. Only two species are known to infect humans through the consumption of meat. They are found in beef and pork and may cause digestive signs (nausea, vomiting enteritis), although most infected persons will show mild or no signs at all. While freezing or cooking of the meat has been shown to kill

sarcocysts, there is no reports of *Sarcocystis* species from marine mammals being harmful to humans (Fayer 2015).

Trematodes have not been reported in beluga lymph node. In Hector's dolphins (*Cephalorhynchus hectori*), an adult trematode had a morphology corresponding to *Campula* spp was noted to be encapsulated in the mesenteric lymph nodes, along with similarly encapsulated aggregates of trematode eggs associated with a strong macrophagic and fibrosing reaction (Hutton 1987). In general, digenean trematode of the genus *Campula* are associated with the liver, bile and pancreatic apparatus in odontocetes. They may cause variable level of lesions and their life cycle is poorly known. In this case, the encapsulated eggs in the lymph nodes were unlikely to have a negative health effect (Measures 2018).

The size of the foetus indicates that this female was bred earlier in the same year. An active lactation could indicate that she had a suckling veal born in the previous summer, or that she was participating in allonursing (Leung 2010).

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes (Lair, 1997).

The lung lesions observed in this individual were *extremely* mild and discrete and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal. The predominant mononuclear cells in conjunction with some eosinophils and focal fibroplasia may indicate a chronic parasitic infection. Lungworms, mainly *Halocercus monoceris*, *H. taurica* and *Stenurus arctomarinus*, are the most frequent cause of parasitic pneumonia in Saint-Lawrence beluga whales (Lair 2016).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

- Fayer R, Esposito DH, Dubey JP. Human infections with *Sarcocystis* species. *Clinical Microbiology Reviews*. 2015 Apr 1;28(2):295-311.
- Hutton J, Blair D, Slooten E, Dawson SM. Case-studies of fluke-induced lesions in mesenteric lymph-nodes of Hector's dolphins *Cephalorhynchus hectori*. *Diseases of Aquatic Organisms*. 1987 Apr 15;2(2):83-6.
- Lair S, Beland P, De Guise S, Martineau D. Adrenal hyperplastic and degenerative changes in beluga whales. *Journal of Wildlife Diseases*. 1997 Jul;33(3):430-7.
- Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. *Veterinary Pathology*. 2016 Jan;53(1):22-36.
- Leung ES, Vergara V, Barrett-Lennard LG. Allonursing in captive belugas (*Delphinapterus leucas*). *Zoo Biology*. 2010 Sep;29(5):633-7.
- Measures LN. Helminths and parasitic arthropods. In: Gulland FM, Dierauf LA, Whitman KL, editors. *CRC Handbook of Marine Mammal Medicine*. CRC Press; 2018. 471-497.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture, Hendrickson Island, 2015-07-16.

Sex: Female

Age: 35

Weight: N/A

Body score (subjective): Good

Length: 379.8 cm

Carcass condition score: 2

External examination

An adult female, mainly white with flippers and fluke of a mild grey color is examined. Two gunshot wounds are seen on each side of the skull.

Internal examination

Uterus: The right horn is twice as large as the left one and contains a male foetus of approximately 20 cm in length.

Lymph nodes: Multiple round and firm nodules of 0,5 to 1,5 cm in diameter, with a necrotic center are found thorough the mesenteric lymph nodes. They are of a normal size.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions: shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, heart, liver, tracheobronchic lymph node, urinary system (bladder, kidneys), spleen.

HISTOPATHOLOGY

Muscle (B, F): Three mature sarcocysts with a thick wall and containing banana shaped merozoites are seen within one section (B). The outer wall of the cyst is indented, delimitating potential septation.

Lymph nodes (D, I): In one section, multifocal discrete accumulations of either neutrophils or eosinophils are found in the cortical region (D). Six 0.5 to 1 cm in diameter nodule are present on a section, with a thick fibrous capsule. Within the nodules, large amounts of degenerate cells, potential degenerate parasites as well as several triangular shaped eggs with a thick pigmented shell are noted (I).

Mammary gland (E): Large portion of the observed section shows inactive glandular tissue. Some epithelial cells and alveoli are gorged with fat droplets and proteinaceous material. Proteinaceous liquid is noted within intralobular canals.

Lungs (J to O): Small, discrete multifocal accumulation of mononuclear cells, generally limited to a single structure (bronchiole, arteriole). In two sections, the infiltration is slightly more extensive and mixed with some eosinophils. A discrete foci of fibroplasia is also noted.

Ovaries (P, Q, R): on one ovary, several follicles at various stages of development/ atresia are noted on the other, a large corpus luteum is noted.

Adrenals (V to AA): Approximately 6 cortical cysts are noted, single or grouped with a characteristic spider web appearance and of 0.5 to 1.5 mm diameter (W).

No significant lesions on the examined lesions from: bladder (A), skin and blubber (A), heart (B), duodenum (C), uterus (C), pancreas (E), liver (F), spleen (G), thyroid (G), kidneys (H), pituitary (S), central nervous system (S, T, U)

Histomorphologic diagnostics

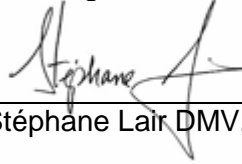
- Mild multifocal muscular sarcocystosis
- Multifocal lymphonodular fibro-necrotic nodular trematodiasis
- Partial lactocyte activity
- Mild eosinophilic bronchopneumonia
- Presence of an ovarian corpus luteum
- Small multifocal adrenocortical cysts

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-16
PATHOLOGY NO.: P2476-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested southeast of Hendrickson Island in Kugmallit Bay on 2015-07-18. It was travelling alone prior to the hunt. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Stenurus* sp., *Halocercus* sp.)**
2. **Muscular sarcocystosis (*Sarcocystis* sp)**
3. **Lymphonodular trematodiasis (probable)**
4. **Gastric nematodiasis (Anisakidae likely)**
5. **Molt**
6. **Inactive spermatogenesis**
7. **Testicular interstitial fibrosis**
8. **Renal medullar and thyroid gland mineralization**
9. **Pituitary cysts and psammoma bodies.**
10. **Adrenocortical cysts**

Interpretation and comments:

None of the conditions noted in this individual was deemed to negatively affect its general condition. This animal's body condition was deemed thin based on available pictures and morphometric measurements; the body mass: total length ratio was of 1.61. However, while total mass and blubber depth appeared inferior to other whales and definition of the head was present, the epaxial muscle (both mass and appearance on pictures) was comparable to individuals attributed with a fair body condition.

The morphology of the parasite observed in the gross examination of the airways is typical of a pseudaliid nematode of the genus *Stenurus* (*S. arctomarinus* has been reported in monodontidae). Pathogeny associated with these lungworms is typically associated to airway occlusion due to the physical presence of parasite within airways. The morphology of the parasite observed in the histopathological examination of the airways as well as associated lesions are typical of an infection with a pseudaliid nematode of the genus *Halocercus* (*H. monoceris* has been reported in monodontidae). Lesions in this individual were generally mild, and the presence of fibroplasia as well as granulomatous reaction are indicative of the chronic parasitic presence. The health of this animal was not affected considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal (Lair 2016, Measures 2018).

The species of sarcocysts found in the muscles is unknown, although we are looking to pursue additional diagnostic. Only two species are known to infect humans through the consumption of meat. They are found in beef and pork and may cause digestive signs (nausea, vomiting enteritis), although most infected persons will show mild or no signs at all. While freezing or cooking of the meat has been shown to kill sarcocysts, there is no reports of *Sarcocystis* species from marine mammals being harmful to humans (Fayer 2015).

Although it was not possible to confirm an etiology in association with the lymph node nodules, trematode eggs have been observed in similar lesions of other beluga whales of this population. Trematodes have not been reported in beluga lymph node. In Hector's dolphins (*Cephalorhynchus hectori*), an adult trematode had a morphology corresponding to *Campula* spp and was noted to be encapsulated in the mesenteric lymph nodes, along with similarly encapsulated aggregates of trematode eggs associated with a strong macrophagic and fibrosing reaction (Hutton 1987). In general, digenean trematode of the genus *Campula* are associated with the liver, bile and pancreatic apparatus in odontocetes. They may cause variable level of lesions and their life cycle is poorly known. In this case, the encapsulated eggs in the lymph nodes were unlikely to have a negative health effect (Measures 2018).

Although morphology of the gastric nematode is lacking, nematodes from the family Anisakidae are found in cetacean stomach. *Anisakis simplex* is the most prevalent gastric helminth in St. Lawrence beluga whales and is usually found in the first compartment of the stomach, while *Contracaecum* sp. were the most prevalent and abundant in beluga whales sampled from the Mackenzie River delta (Lair 2016, Measures 1995, Wazura 1986).

The histology of the skin lesions observed in this animal are consistent with a normal molt process. Similar changes were noted in the spring migration as well as in the summer estuarine passage of Hudson Bay beluga whales (St. Aubin 1990).

The scarce presence of spermatozooids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989). Interstitial fibrosis is likely a normal process associated with age (Foster 2016).

Pituitary cysts and psammoma bodies have been described in cetacean species (Cowan 2008) and this observation is not considered to be significant, such as the small amount of mineralization noted in the kidneys and thyroid gland.

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes (Lair, 1997).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

- Cowan DF, Haubold EM, Tajima Y. Histological, immunohistochemical and pathological features of the pituitary gland of odontocete cetaceans from the western Gulf of Mexico. *Journal of Comparative Pathology*. 2008 Aug 1;139(2-3):67-80.
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- Hutton J, Blair D, Slooten E, Dawson SM. Case-studies of fluke-induced lesions in mesenteric lymph-nodes of Hector's dolphins *Cephalorhynchus hectori*. *Diseases of Aquatic Organisms*. 1987 Apr 15;2(2):83-6.
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- Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. *Veterinary Pathology*. 2016 Jan;53(1):22-36.
- Measures LN, Béland P, Martineau D, Guise SD. Helminths of an endangered population of belugas, *Delphinapterus leucas*, in the St. Lawrence estuary, Canada. *Canadian Journal of Zoology*. 1995 Aug 1;73(8):1402-9.
- Measures LN. Helminths and parasitic arthropods. In: Gulland FM, Dierauf LA, Whitman KL, editors. *CRC Handbook of Marine Mammal Medicine*. CRC Press; 2018. 471-497.
- O'Brien JK, Steinman KJ, Schmitt T, Robeck TR. Semen collection, characterisation and artificial insemination in the beluga (*Delphinapterus leucas*) using liquid-stored spermatozoa. *Reproduction, Fertility and Development*. 2008 Oct 6;20(7):770-83.
- St Aubin DJ, Smith TG, Geraci JR. Seasonal epidermal molt in beluga whales, *Delphinapterus leucas*. *Canadian Journal of Zoology*. 1990 Feb 1;68(2):359-67.
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- Wazura KW, Strong JT, Glenn CL, Bush AO. Helminths of the beluga whale (*Delphinapterus leucas*) from the Mackenzie River Delta, Northwest Territories. *Journal of Wildlife Diseases*. 1986 Jul;22(3):440-2.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture, Hendrickson Island, 2015-07-18.

Sex: Male

Age: 39

Weight: 693 kg *(estimation, tail still a little bit in water)

Body score (subjective): Excellent

Length: 429.4 cm

Carcass condition score: 2

External examination

An adult white male is examined. The harpoon is located on the caudo-dorsal right epaxial muscles. Three gunshot wounds are noted on the right side of the head and thorax. Occasional circular depressions 1 to 2 cm in diameter are found on the skin surface.

Internal examination

Lungs: A small number of nematodes of 1x 50 mm in size is found in the cranial and caudal portions of both lungs. A clear to brown in color mucous material is found in some smaller airways. Both lungs appear over inflated.

Lymph nodes: Rare round and firm nodules of 0,5 to 1 cm in diameter, with a necrotic center are found thorough the mesenteric lymph nodes. The mesenteric lymph nodes are of a normal size.

Stomach and intestines: A white nematode, as well as a dozen squid beaks are found in the first gastric compartment. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions; shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, heart, liver, tracheobronchic lymph node, urinary system (bladder, kidneys), spleen.

HISTOPATHOLOGY

Skin (A): In one of the two sections observed, cells from the most superficial skin later are polyhedral, irregularly arranged and sometimes vacuolized. These degenerate cells are partially torn away from the underlying cells. Underlying progressive flattening of the epithelial cells from the stratum intermedium indicates formation of a new stratum externum.

Muscle (D): A single 0.1 mm in diameter sarcocyst is found in a muscle fiber on one of two sections evaluated. The cyst is enveloped by a relatively thin capsule and contains banana shaped merozoites. The center of the cyst is vacuolated.

Thyroid (E, F): There is a marked difference in thyroid follicular sizes, as well as a discrete mineralization foci.

Lymph node (F, G, H): A single thick fibrous cysts is noted. The central portion of the cysts consists of a necrotic center composed of degenerated inflammatory cells and mineralisation foci.

Reproductive tract (H, I): No sperm is stored in the epididymis (H), and there is no significant spermatogenesis within the testis (I). Diffuse, moderate interstitial fibrosis is noted in the testis.

Pancreas (I): A discrete nodular, non-encapsulated region of pancreatic exocrine hyperplasia is noted. Acinar cells appear mildly enlarged in this section.

Kidneys (J): Multifocal, discrete mineralization foci within medullar collecting tubules are seen in one renicule.

Pituitary gland (Q): Multifocal aggregates of small punctual mineralization foci are found within the hypophysis. Aggregates consisting of multiple enlarged follicles, as well as occasional cysts (n=4) of 0.5 to 1 mm in diameter are also noted.

Lungs (K to P): Sections of nematodes (<0.1 mm diameter) in a clear bronchiole are noted (K-m). Mild, discrete relatively diffuse areas of mononuclear inflammatory cell (histiocytes / lymphoplasmocytic) infiltration are noted and may be associated with the presence of eosinophils and alveolar macrophages in associated airways (mostly alveolar). Also, small areas of interstitial lymphoplasmacytic infiltration, associated with mild fibroplasia resulting in focal fusion of alveolar walls and presence of a few eosinophils within alveolar lumen (L-s) or intralesional proteinaceous debris and multinucleated giant cells (L-m) are noted. Focally, a granulomatous reaction of 1 mm in diameter

centered on a bronchiole with a necrotic, partially mineralized lumen is noted (P-m). Rare clusters of alveoles are filled with proteinaceous droplets with scarce alveolar macrophages (M-p)

Adrenals glands (S-X): Closely clustered adrenocortical cysts of up to 0.1x 0.3 mm diameter are noted (T)

No significant lesions on the examined lesions from: heart (B), duodenum (C), bladder (C), lymph node (C), liver (E), spleen (E), central nervous system (Q, R).

Histomorphologic diagnostics

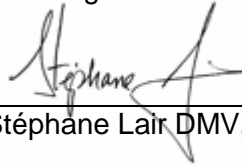
- Desquamation of degenerate epidermal cell layer and formation of a new stratum externum.
- Focal muscular sarcocystosis
- Discrete mineralization within the thyroid gland
- Lymphonodular fibro-necrotic nodule
- Inactive spermatogenesis and interstitial fibrosis
- Multifocal discrete renal medullar mineralization
- Multifocal pituitary cysts and psammoma bodies.
- Mild multifocal eosinophilic interstitial pneumonia and fibroplasia. Focal granulomatous bronchopneumonia and focal nematodiasis (*Halocercus* sp.).
- Adrenocortical cysts

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-17
PATHOLOGY NO.: P2477-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested in Kugmallit Bay on 2015-07-18. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Halocercus* sp.)**
2. **Renal nematodiasis (*Crassicauda giliakiana*)**
3. **Lymphonodular trematodiasis (probable)**
4. **Molt**
5. **Inactive spermatogenesis**
6. **Testicular interstitial fibrosis**
7. **Thyroid nodular hyperplasia**
8. **Multifocal renal medullar and epididymal epithelium mineralization**
9. **Multifocal pituitary cysts and psammoma bodies.**

Interpretation and comments:

None of the conditions noted in this individual was deemed to negatively affect its general condition. This animal's body condition was deemed fair based on limited available pictures and morphometric measurements.

The morphology of the parasite observed in the airways as well as associated lesions are typical of an infection with a pseudaliid nematode of the genus *Halocercus* (*H. monoceris* has been reported in monodontidae). Multifocal active foci of inflammation were noted in this individual, while the presence of fibroplasia as well as granulomatous reaction are indicative of the chronic presence of this parasite. The health of this animal was not affected considering the absence of gross lesions, the relatively small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal (Lair 2016, Measures 2018).

The location and morphology of the encapsulated renal nematode is typical of the genus *Crassicauda* sp, a spirurid nematode found in mysticetes and odontocetes. *Crassicauda giliakiana* is generally site specific and associated to the urogenital system (Measures 2018). Although significant lesions can sometimes be associated with this parasite, in this animal the small proportion of renal tissue affected did not negatively impact its health.

Although it was not possible to confirm an etiology in association with the lymph node nodules, trematode eggs have been observed in similar lesions of other beluga whales of this population. In Hector's dolphins (*Cephalorhynchus hectori*), an adult trematode had a morphology corresponding to *Campula* spp was noted to be encapsulated in the mesenteric lymph nodes, along with similarly encapsulated aggregates of trematode eggs associated with a strong macrophagic and fibrosing reaction (Hutton 1987). In general, digenean trematode of the genus *Campula* are associated with the liver, bile and pancreatic apparatus in odontocetes. They may cause variable level of lesions and their life cycle is poorly known. In this case, the encapsulated eggs in the lymph nodes were unlikely to have a negative health effect (Measures 2018).

The histology of the skin lesions observed in this animal are consistent with a normal molt process. Similar changes were noted in the spring migration as well as in the summer estuarine passage of Hudson Bay beluga whales (St. Aubin 1990).

The scarce presence of spermatozooids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989). Interstitial fibrosis is likely a normal process associated with age (Foster 2016).

Thyroid adenomatous hyperplasia have been described in both arctic (Hudson Bay) and St. Lawrence beluga populations, where the total volume of lesions was positively correlated with age (Mikaelian 2003).

Pituitary cysts and psammoma bodies have been described in cetacean species (Cowan 2008) and this observation is not considered to be significant, such as the small amount of mineralization noted in the kidneys and epididymis.

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

- Cowan DF, Haubold EM, Tajima Y. Histological, immunohistochemical and pathological features of the pituitary gland of odontocete cetaceans from the western Gulf of Mexico. *Journal of Comparative Pathology*. 2008 Aug 1;139(2-3):67-80.
- Foster RA. Male genital system. In Maxie MG Ed. *Jubb, Kennedy & Palmer's Pathology of Domestic Animals*. 6th ed. Missouri, USA: Elsevier; 2016. V.3 p.465-510.
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- Stewart BE, Stewart RE. *Delphinapterus leucas*. *Mammalian Species*. 1989; (336): 1-8.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture and Sonia Ostertag, PhD on Hendrickson Island, 2015-07-18.

Sex: Male

Age: 25

Weight: N/A

Body score (subjective): Fair

Length: 409 cm

Carcass condition score: 2

External examination

An adult white male is examined. The harpoon was located on the left dorsum, at the anal level. Occasional circular depressions 1 to 2 cm in diameter are found on the skin surface.

Internal examination

Adrenal glands: The right adrenal gland is half the normal size.

Lymph nodes: Multiple round and firm nodules of 0,5 to 1 cm in diameter, with a necrotic center are found thorough the mesenteric lymph nodes. The mesenteric lymph nodes are of a normal size.

Kidney: A 3 cm in diameter cyst is present in the right kidney. A 3x150 mm long nematode is also present in the kidney.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions: shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, lungs, heart, liver, tracheobronchic lymph node, urinary system (bladder, kidneys), spleen.

HISTOPATHOLOGY

Skin and blubber (A): There is a superficial tear of the stratum externum. A cluster of these cell is polyhedral. The underlying cell layers are normal in appearance.

Reproductive tract (C, E): No sperm is stored in the epididymis (E), and there is no significant spermatogenesis within the testis, with a segment where spermatocytes appear atrophied (C). Diffuse, moderate interstitial fibrosis is noted in the testis. Multifocally, clusters of acidophilic cells are seen within spermatid tubules. There is multifocal, punctual foci of mineralisation within the epididymal epithelium.

Lymph nodes (D, E, S, T, U): Aggregation of eosinophils are sometimes seen in cortical lymphoid centers (D, E). Several nodules, composed of thick layers of fibrous tissue composed and a necrotic center with various amounts of degenerated cells, granular mineralized material, and disorganized conjunctive tissue are noted. These nodules range from 1 mm to 15 mm in diameter.

Thyroid (F, R): Four sections are examined. The follicles are relatively uniform in size. Some larger follicles of approximately 1 mm in diameter are noted. A focal, 1 mm foci of nodular hyperplasia is also noted (F).

Pancreas (F): The pancreas acini appearance is slightly heterogenous. Multifocally, acinic cells appear paler and larger in association with a foamy cytoplasm.

Kidneys (H, I): In a renicular medulla, multifocal punctual aggregation of granular, mineralized material are seen within small blood vessels (H). A thick fibrous capsule is noted on the other section examined, with no associated inflammatory reaction.

Lungs (J to O): multifocal mild to marked interstitial infiltration of mononuclear inflammatory cells, sometimes associated with eosinophils. Also, multifocal, mild to marked intraluminal alveolar and bronchiolar aggregations of eosinophils and alveolar macrophages, sometimes associated with large numbers of erythrocytes are seen. While some lesions are relatively focal and discrete, interstitial infiltration is sometimes extensive and not well defined. Lesions of combined interstitial and intraluminal inflammatory cell infiltration are usually focally expansive. Areas of fibroplasia, with fibrocytes, slightly thickened, sometimes coalescent alveolar walls and no apparent capillaries are also noted (L-s). Within the airway of a markedly affection region, cut sections of a small, less than 0.1 mm in diameter nematode are noted (K-m). Focally, a 0.5 mm granuloma with a mineralized center is noted (M-m).

Pituitary gland (Q): Multifocal aggregates of small punctual mineralization foci are found within the pituitary. One cyst of 0.5 mm in diameter is noted and contains a dark acidophilic colloid. Two large follicles of approximately 0.1 mm in diameter are noted nearby.

Adrenals (V to X): A single gland is examined. Nothing abnormal.

No significant lesions on the examined lesions from: bladder (A), heart (B), muscle (B), duodenum (C), spleen (G), liver (G), central nervous system (P,Q), adrenal (V to X)

Histomorphologic diagnostics

- Desquamation of degenerate epidermal cell layer and formation of a new stratum externum.
- Inactive spermatogenesis
- testicular interstitial fibrosis

- Epididymal epithelium multifocal discrete mineralization
- Multifocal lymphonodular fibro-necrotic nodules
- Focal thyroid nodular hyperplasia
- Focal renal fibrous cyst
- Multifocal renal medullar mineralization
- Multifocal pituitary cysts and psammoma bodies.
- Mild multifocal eosinophilic, verminous (*Halocercus* sp.) bronchopneumonia and fibroplasia.
Focal granulomatous bronchopneumonia.

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-19
PATHOLOGY NO.: P2478- 15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested southwest of Hendrickson Island in Kugmallit Bay on 2015-07-19. It was in a group of three white whales. The group appeared to be travelling and feeding prior to the hunt. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous pneumonia (*Stenurus* sp., *Halocercus* sp. likely)**
2. **Multifocal muscle sarcocystosis**
3. **Adrenocortical cysts**
4. **Thyroid nodular hyperplasia**
5. **Inactive spermatogenesis**
6. **Pituitary cysts and psammoma bodies**
7. **Renal and epididymal mineralization**
8. **Molt**
9. **Mild dermatitis, unknown etiology**
10. **Gastric nematodiasis (*Anisakidae* likely)**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed good based on available pictures and morphometric measurements; He had one of the most important epaxial muscle weight recorded. On available pictures, the skin surface cranial to the dorsal crest almost appears straight and the lumbar dorsal epaxial musculature appears well developed.

The nematodes observed upon gross examination of the lungs, as well as the smaller larvae observed within the histologic examination of the lung correspond to the genus *Stenurus* sp. Pathogeny with this genus is usually associated with the physical blockade of airway, but a role in some of the inflammatory reaction cannot be excluded. The described lung lesions, including granulomas with a mineralized center, are typical of an infection with a pseudaliid nematode of the genus *Halocercus* (*H. monoceris* has been reported in monodontidae). Multifocal active foci of inflammation were noted in this individual, while the presence of fibroplasia as well as granulomatous reaction are indicative of the chronic presence of this parasite. The health of this animal was not affected considering the rare gross lesions observed, the relatively small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal (Lair 2016, Measures 2018).

The species of sarcocysts found in the muscles is unknown, although we are looking to pursue additional diagnostic. Only two species are known to infect humans through the consumption of meat. They are found in beef and pork and may cause digestive signs (nausea, vomiting enteritis), although most infected persons will show mild or no signs at all. While freezing or cooking of the meat has been shown to kill sarcocysts, there is no reports of *Sarcocystis* species from marine mammals being harmful to humans (Fayer 2015).

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes (Lair, 1997).

Thyroid adenomatous hyperplasia has been described in both arctic (Hudson Bay) and St. Lawrence beluga populations. The volume of adenomatous hyperplasia was positively correlated with age (Mikaelian 2003).

The scarce presence of spermatozoids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

Pituitary cysts and psammoma bodies have been described in cetacean species (Cowan 2008) and this observation is not considered to be significant, such as the small amount of mineralization noted in the kidneys and epididymis.

The histology of the skin lesions observed in this animal are consistent with a normal molt process. Similar changes were noted in the spring migration as well as in the summer estuarine passage of Hudson Bay beluga whales (St. Aubin 1990). The observed dermatitis was mild, likely non clinically significant considering the lack of gross lesion description and of an unknown etiology based on observed sections.

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

- Cowan DF, Haubold EM, Tajima Y. Histological, immunohistochemical and pathological features of the pituitary gland of odontocete cetaceans from the western Gulf of Mexico. *Journal of Comparative Pathology*. 2008 Aug 1;139(2-3):67-80.
- Fayer R, Esposito DH, Dubey JP. Human infections with *Sarcocystis* species. *Clinical Microbiology Reviews*. 2015 Apr 1;28(2):295-311.

- Lair S, Beland P, De Guise S, Martineau D. Adrenal hyperplastic and degenerative changes in beluga whales. *Journal of Wildlife Diseases*. 1997 Jul;33(3):430-7.
- Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. *Veterinary Pathology*. 2016 Jan;53(1):22-36.
- Measures LN. Helminths and parasitic arthropods. In: Gulland FM, Dierauf LA, Whitman KL, editors. *CRC Handbook of Marine Mammal Medicine*. CRC Press; 2018. 471-497.
- Mikaelian I, Labelle P, Kopal M, De Guise S, Martineau D. Adenomatous hyperplasia of the thyroid gland in beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary and Hudson Bay, Quebec, Canada. *Veterinary Pathology*. 2003 Nov;40(6):698-703.
- O'Brien JK, Steinman KJ, Schmitt T, Robeck TR. Semen collection, characterisation and artificial insemination in the beluga (*Delphinapterus leucas*) using liquid-stored spermatozoa. *Reproduction, Fertility and Development*. 2008 Oct 6;20(7):770-83.
- St Aubin DJ, Smith TG, Geraci JR. Seasonal epidermal molt in beluga whales, *Delphinapterus leucas*. *Canadian Journal of Zoology*. 1990 Feb 1;68(2):359-67.
- Stewart BE, Stewart RE. *Delphinapterus leucas*. *Mammalian Species*. 1989; (336): 1-8.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture, Hendrickson Island, 2015-07-19.

Sex: Male

Age: 34

Weight: N/A

Body score (subjective): good

Length: 421.8 cm

Carcass condition score: 2

External examination

An adult white male is examined. A harpoon is located between the dorsal crest and the anal region, on the left dorsum. Several gunshot wounds are noted on the right thoracic region (4) and on the neck (1). Occasional circular depressions 1 to 2 cm in diameter are found on the skin surface.

Internal examination

Thyroid gland: A 1 cm in diameter cyst is found in the thyroid parenchyma.

Lungs: A mild amount of nematodes, black and white in coloration and 1x 50 mm in size are found in the airways of the medial part of both lungs. In the superficial dorsal portion of the right lung, an irregular region of indurated parenchyma of approximately 4x5 cm is noted.

Stomach and intestines: Rare nematodes are found in the stomach's first compartment. No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions; shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, lungs, heart, liver, lymph nodes (tracheobronchic and mesenteric), urinary system (bladder, kidneys), spleen.

HISTOPATHOLOGY

Skin (A, B): One of the sections examined is normal (A). On the other section on A, there is mild, multifocal mononuclear (histiocytic) cell infiltration of the dermal papillae, but not extending in the dermis. The most superficial cells are polygonal, sometimes vacuolated and partially sloughed off. Similar epidermal observation (polygonal, vacuolated cells and sloughing of degenerate cells) are noted on the superficial epidermal section on B, especially surrounding the crateriform thinning of the epidermal layer. The stratum externum thickness is irregular. On B (tongue), the skin appears normal, albeit from another body region considering the different architecture including smaller overall thickness and a thin dermal layer rich in connective tissue and glands but with rare adipose cells

Muscle (C, B, D): On two sections, a cystic, encapsulated and trabeculated structure is noted in a muscular fiber and contains numerous small and elongated (banana shaped) basophil structures (merozoites) (B- tongue,D). Septations are partially vacuolated. Occasional coagulated and fragmented cells are noted in the subcutaneous muscle (D).

Thyroid (J, G): A 1 cm in diameter mass is noted in the thyroid tissue. It is surrounded by a thin fibrous capsule and compresses the adjacent thyroid gland. The mass is highly cellular and formed by numerous densely packed and homogenous epithelial cells, often organised in thyroidal lobules of various size and shape. Some of these lobules are distended (cystic). Cysts are filled with dense colloid. No mitosis figures are noted.

Reproductive tract (H): No sperm is stored in the epididymis, and there is no significant spermatogenesis within the testis. There is multifocal, punctual foci of mineralisation within the epididymal epithelium.

Kidney (K): A single mineralization foci is noted within a blood vessel in a renicular medulla.

Lungs (L to Q+ F): Mild, discrete to diffuse interstitial infiltration of mononuclear cells (histiocytes), sometimes associated with fibroblasts and/or eosinophils is seen in the alveolar wall, as well as in the periphery of arterioles, bronchioles and final bronchi (N-m). Multinucleated giant cells as well as radiating, granular acidophilic material may be seen within lesions (L-p, M-s). Fibroplasia, along with chronic inflammatory reaction may locally create airway coalescence and obliterate alveolar capillaries. In sections where interstitial lesions are more locally extensive, the eosinophil proportion is usually greater and adjoining alveolar and bronchiolar lumen may be filled with eosinophils and alveolar macrophages, as well as varying numbers of erythrocytes (M-s). Sections of nematodes, 0.15 mm in diameter are noted within airways without inflammatory reaction (O-s, *Stenurus* sp).

In the additional section from macroscopically affected parenchyma (F), multiple granulomas, of 0.5 to 1 mm in diameter, variably and partially encapsulated with fibrous tissue and with a necrotic and or mineralized center are noted. Interstitial infiltration of mononuclear cells and eosinophils radiate extensively in the parenchyma surrounding these lesions, and a lot of airways are filled with proteinaceous liquid, some alveolar macrophages and scarce eosinophils.

Pituitary gland (R): An aggregate of slightly distended ductules with mineralized material in the lumen is noted in the adenohypophysis.

Adrenal (T to Y): Four cortical cysts, of approximately 0.50 mm in diameter are noted (T, V). They are lined with a flat to cuboidal epithelium and filled with colloid.

No significant lesions on the examined lesions from: heart (C), Bladder* (E), duodenum (E), lymph nodes (E, F, H), spleen (J), pancreas (I), liver (I), central nervous system (S, R).

Histomorphologic diagnostics

- Desquamation of degenerate epidermal cell layer
- Mild histiocytic dermatitis
- Multifocal muscle sarcocystosis
- Thyroid nodular hyperplasia
- Inactive spermatogenesis

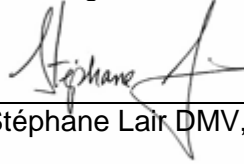
- Renal medulla and epididymal multifocal mineralization
- Mild to moderate multifocal eosinophilic interstitial pneumonia and fibroplasia. Multifocal eosinophilic and granulomatous bronchopneumonia and focal nematodiasis (*Stenurus* sp., *Halocercus* sp. possible).
- Hypophysis multifocal psammoma bodies
- Multifocal small adrenocortical cysts

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair, DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-20
PATHOLOGY NO.: P2479-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested south of Hendrickson Island in Kugmallit Bay on 2015-07-19. It was in a group of three white whales that appeared to be feeding prior to the hunt. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Stenurus* sp. *Halocercus* sp. possible)**
2. **Muscular sarcocystosis**
3. **Inactive spermatogenesis**
4. **Focal myositis**
5. **Adrenocortical cysts**
6. **Molt**
7. **Focal myositis**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was not evaluated as neither pictures, total body mass or epaxial muscle mass are available. However, ½ girths measured suggest he had a fairly good body condition when compared to other whales.

The morphology of the parasite observed during gross examination of the lungs is typical of the pseudaliid nematode of the genus *Stenurus* (*S. arctomarinus* has been reported in monodontidae). Pathogeny

associated with these lungworms is typically associated to airway occlusion due to the physical presence of parasite within airways. The parasite was not observed histologically and its role in the observed lesions remains undetermined. Alternatively, or additionally, the mineralized structure in a granulomatous center could correspond to the remain of a pseudaliid nematode of the genus *Halocercus* sp., as the size and described lesions would correspond to reported lesions in association with this parasite. Despite these observations, the lung lesions in this individual were mild and did not affect its health considering the rare gross lesions (indured parenchyma), the generally chronic and mostly inactive lesions and small proportion of affected parenchyma on microscopic examination of the lung as well as the good general condition of the animal (Lair 2016, Measures 2018).

The species of sarcocysts found in the muscles is unknown, although we are looking to pursue additional diagnostic. Only two species are known to infect humans through the consumption of meat. They are found in beef and pork and may cause digestive signs (nausea, vomiting enteritis), although most infected persons will show mild or no signs at all. Freezing or cooking of the meat is recommended, as it has been shown to kill sarcocysts (Fayer 2015).

The scarce presence of spermatozoids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes (Lair, 1997).

The histology of the skin lesions observed in this animal are consistent with a normal molt process. Similar changes were noted in the spring migration as well as in the summer estuarine passage of Hudson Bay beluga whales. The dorsal localisation of these lesions suggests may be consequent with recent passage and skin abrasion under ice sections (St. Aubin 1990).

The clinical signification, cause, or tissue of origin for the focal myositis noted are uncertain.

Please note that lesions deemed associated with the harvesting method are not described in this report.

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- Lair S, Beland P, De Guise S, Martineau D. Adrenal hyperplastic and degenerative changes in beluga whales. *Journal of Wildlife Diseases*. 1997 Jul;33(3):430-7.
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DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture and Sonia Ostertag, PhD on Hendrickson Island, 2015-07-19.

Sex: Male

Age: 27

Weight: ND

Body score (subjective): ND

Length: 414.1 cm

Carcass condition score: 2

External examination

An adult white male is examined. Occasional circular depressions 1 to 2 cm in diameter are found on the skin surface.

Internal examination

Lungs: Occasional nematodes, black and white in coloration and 1x 50 mm in size are found in the airways of the medial part of both lungs. In the superficial dorsal portion of the right lung, an irregular region of indurated parenchyma of approximately 4x5 cm is noted.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions; shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, lungs, heart, liver, lymph nodes (tracheobronchic and mesenteric), urinary system (bladder, kidneys), spleen.

HISTOPATHOLOGY

Skin (A, B): On one section, the parakeratotic layer is mildly irregular and condensed. Small mineral corpuscles as well as unicellular organisms are nested in this irregular outer portion. The unicellular organisms, presumably algae, are sometimes infiltrating cells.

Tongue (B): This transverse cut is of a tissue of 2 cm in thickness with a relatively thin, stratified, non-keratinized squamous epithelium on both side and a thin dermis with dense conjunctive tissue flanking a medial muscular layer. Within this muscular layer, a whole muscle section is replaced by a mononuclear inflammatory cell infiltration.

Muscle (D): Approximately 10 cyst-like structures are noted in a few muscular fibers and contain numerous small and elongated, banana-shaped basophil structures (merozoites). Some sarcocysts are immature and metrocytes may be seen within.

Reproductive tract (G): No sperm is stored in the epididymis, and there is no significant spermatogenesis within the seminiferous tubules. There are multifocal, punctual foci of mineralisation within the epididymal epithelium.

Lymph node (F, G): Multifocal small foci of neutrophil and eosinophil aggregates surrounded by macrophages are noted. Proteinaceous, radiated material is sometimes noted within those aggregates

(Splendore-hoeppli bodies). No bacteria or alchoolo-acido resistant bacilli are noted on Gram (Brown-Hopps) and Ziehl-Neelson stains, respectively.

Lungs (K to P): Mild to moderate, multifocal, discrete to relatively diffuse areas of mononuclear cell infiltration, sometimes associated with fibroplasia or eosinophils are noted (notably: N-m). Focally (k-p), a granulomatous reaction is centered on a mineralized center. Focally (O-m), a nodular inflammatory cell infiltration process associated with a bronchiole is centered on degenerated cells and multinucleated giant cells. In rare affected sections (O-p), airways associated with interstitial infiltration are also filled with erythrocytes, eosinophils and alveolar macrophages.

Adrenals (S to X): Rare cysts of a diameter less than 0.36 mm are noted (V)

No significant lesions on the examined lesions from: Bladder (B), duodenum (E), heart (C), liver (I), pancreas (H), spleen (H), thyroid (I), kidney (J), cerebrum (R), cerebellum (Q), medulla oblongata (R), pituitary gland (Q).

Histomorphologic diagnostics

- Superficial degeneration of the stratum externum
- Focal histiocytic myositis
- Multifocal muscular sarcocystosis
- Inactive spermatogenesis
- Multifocal lymphonodular eosinophil/neutrophil aggregation with splendore-hoeppli bodies
- Mild multifocal interstitial eosinophilic bronchopneumonia and fibrosis
- Adrenocortical cysts

Resident:

Émilie L. Couture, DMV, IPSAV, DES

Pathologist:

Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-21
PATHOLOGY NO.: P2480-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested west of Hendrickson Island in Kugmallit Bay on 2015-07-19. It was in a group of six to ten whales that included dark, grey and white whales. The group appeared to be travelling north prior to the hunt. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Stenurus* sp., *Halocercus* sp.)**
2. **Lymphonodular trematodiasis**
3. **Inactive spermatogenesis**
4. **Focal lymphoplasmacytic pancreatitis**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed fair based on available pictures and morphometric measurements. Body mass: total length ratio was 1.66, which is intermediate between thin and fair individuals and epaxial muscle weight as well as ½ girths are relatively small in this short male. However, based on available pictures the individuals would probably have been qualified as having a fair body condition.

The morphology of the parasite observed during gross examination of the lungs are typical of an infection with a pseudaliid nematode of the genus *Stenurus* (*S. arctomarinus* has been reported in monodontidae). The morphology of the parasite observed in on histological sections as well as associated lesions are typical of an infection with a pseudaliid nematode of the genus *Halocercus* (*H. monoceris* has been

reported in monodontidae). Lesions in this individual were generally mild, and the presence of fibroplasia as well as granulomatous reaction are indicative of the chronic parasitic presence. Despite some more heavily affected sections, with proportion of affected parenchyma sometimes reaching 60%, the health of this animal was not affected considering rare gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal (Lair 2016, Measures 2018).

Trematodes have not been reported in beluga lymph node. In Hector's dolphins (*Cephalorhynchus hectori*), an adult trematode had a morphology corresponding to *Campula* spp was noted to be encapsulated in the mesenteric lymph nodes, along with similarly encapsulated aggregates of trematode eggs associated with a strong macrophagic and fibrosing reaction (Hutton 1987). In general, digenean trematode of the genus *Campula* are associated with the liver, bile and pancreatic apparatus in odontocetes. They may cause variable level of lesions and their life cycle is poorly known. In this case, the encapsulated eggs in the lymph nodes were unlikely to have a negative health effect (Measures 2018).

The scarce presence of spermatozoids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

- Hutton J, Blair D, Slooten E, Dawson SM. Case-studies of fluke-induced lesions in mesenteric lymph-nodes of Hector's dolphins *Cephalorhynchus hectori*. Diseases of Aquatic Organisms. 1987 Apr 15;2(2):83-6.
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- O'Brien JK, Steinman KJ, Schmitt T, Robeck TR. Semen collection, characterisation and artificial insemination in the beluga (*Delphinapterus leucas*) using liquid-stored spermatozoa. Reproduction, Fertility and Development. 2008 Oct 6;20(7):770-83.
- Stewart BE, Stewart RE. *Delphinapterus leucas*. Mammalian Species. 1989; (336): 1-8.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Stéphane Lair and Dr. Émilie L. Couture, Hendrickson Island, 2015-07-19.

Sex: Male

Age: 23

Weight: 624 kg

Body score (subjective): Fair

Length: 376 cm

Carcass condition score: 2

External examination

An adult white male is examined. The harpoon is located caudally and to the left of the dorsal crest. Several gunshot wounds (4-5) are noted on the left thoracic and neck regions. Occasional circular depressions 1 to 2 cm in diameter are found on the skin surface.

Internal examination

Lungs: A moderate number of nematodes, black and white in coloration and 1x 50 mm in size are found in both lungs. Half a dozen region of irregular parenchymal induration of approximately 1.5 to 2.5 cm and beige in color are noted in the lungs

Lymph nodes: Multiple round and firm nodules of 0,5 to 1 cm in diameter, with a necrotic center are found in approximately half the mesenteric lymph nodes. The mesenteric lymph nodes are of a normal size.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions: shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, heart, liver, lymph nodes (tracheobronchic), urinary system (bladder, kidneys), spleen.

HISTOPATHOLOGY

Reproductive tract (G, H): No sperm is stored in the epididymis (G), and there is no significant spermatogenesis within the seminiferous tubules (H).

Lymph node (C, E, F, G, H): Germinal centers are prominent in the lymph nodes. Locally extensive, mild infiltration of eosinophils and mononuclear cells can be seen in trabeculae and capsule (C, H). On a different section (F), a thick fibrous capsule is centered on degenerate mineralized material that contains necrotic inflammatory cells and a section of a degenerate trematode. Within the trematode, characteristic triangle shaped eggs with thick pigmented walls are seen. The fibrous capsule is infiltrated with macrophages laden with golden pigments. Similar necrotic centers surrounded by a fibrous capsule are noted on slides G and E, and trematodes eggs can be noted within the necrotic material (E).

Lungs (J to O): Multifocal interstitial inflammatory cells (histiocytes, eosinophils) infiltration is noted and may affect various parts of the parenchyma, such as alveolar walls, bronchioles, perivascular spaces and subcapsular area (J-s). Lesions may be associated with proteinaceous radiated material and multinucleated giant cells (J-s, M-p, O-s), with cut sections of nematodes approximately 0.1mm in diameter (M-p) or be focally extensive and associated with nodular lamellar aggregations of histiocytes and collagen fibers with a center composed of necrotic cellular debris, proteinaceous material, neutrophils and mineralized particles (K-s). Some of these areas, usually more extensive, are associated with the intraluminal presence of eosinophils, alveolar macrophages and erythrocytes. In the most affected section (K-s), most airways in the affected region and peripheral to the granulomas are filled with a proteinaceous liquid with some eosinophils and alveolar macrophages. In affected regions with mostly histiocytic infiltrations, lesions are focal and discrete, or relatively diffuse and mild. Varying degrees of fibroplasia may be associated with these regions: alveolar architecture may appear modified, with irregular, sometimes coalescent alveolar walls and non-apparent aerial capillaries (K-m), or large areas of collagenous deposition associated with the presence of fibrocytes may be seen, focally obliterating lung architecture and coursed by multiple small blood vessels (M-s). Adjacent groupment of alveoli may be distended with air and droplets of eosinophilic liquid (M-s). In the cranial portion of the right lung (J-m+p), alveoli are distended by substantial amount of blood.

No significant lesions on the examined lesions from: Skin (A), bladder (A), heart (B), muscle (B, D), spleen (C), pancreas (C), thyroid (D), liver (D), kidney (I), duodenum (I), cerebrum (P), cerebellum (P), medulla oblongata (Q), pituitary gland (Q), adrenals (R to W)

Histomorphologic diagnostics

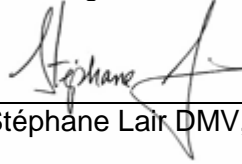
- Inactive spermatogenesis
- Multifocal encapsulated lymphonodular trematodiasis
- Multifocal, mild to moderate interstitial eosinophilic and histiocytic verminous bronchopneumonia, focally extensive necro-suppurative granulomas and multifocal mild fibroplasia (*Halocercus* sp.)

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair, DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2015-22
PATHOLOGY NO.: P2481-15

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested south of Hendrickson Island in Kugmallit Bay on 2015-07-26. It was alone. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Stenurus* sp., *Halocercus* sp. probable)**
2. **Lymphonodular encapsulated trematodiasis**
3. **Mild senile atrophy of the brain**
4. **Thyroid follicular cysts and nodular hyperplasia**
5. **Inactive spermatogenesis**
6. **Focal lymphoplasmacytic orchitis**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed excellent based on available pictures and morphometric measurements. The epaxial muscle mass was important in this large individual.

The morphology of the larger parasite observed upon gross and microscopic evaluation of the lungs is typical of the pseudaliid nematode *Stenurus* sp. (*S. arctomarinus* has been reported in monodontidae). Some of the extensive lesions reported in this individual seem to be attributable to the locally extensive bronchopneumonia caused by the presence of this parasite. In addition to the definitive presence of *Stenurus* sp., what appears to be the shape of a degenerated nematode of the size of *Halocercus*,

another pseudaliid nematode associated with verminous pneumonia in cetaceans has been observed (*H. monoceris* has been reported in monodontidae). Lesions in this individual were more pronounced in the left lung, were lesions were generally relatively extensive, whether active or chronic in nature. Despite some more heavily affected sections, the health of this animal was not affected considering the scarce lesions observed on gross necropsy and the good general condition of the animal (Lair 2016, Measures 2018).

Trematodes have not been reported in beluga lymph node. In Hector's dolphins (*Cephalorhynchus hectori*), an adult trematode had a morphology corresponding to *Campula* spp and was noted to be encapsulated in the mesenteric lymph nodes, along with similarly encapsulated aggregates of trematode eggs associated with a strong macrophagic and fibrosing reaction (Hutton 1987). In general, digenean trematode of the genus *Campula* are associated with the liver, bile and pancreatic apparatus in odontocetes. They may cause variable level of lesions and their life cycle is poorly known. In this case, the encapsulated eggs in the lymph nodes were unlikely to have a negative health effect (Measures 2018).

The presence of macrophages laden with lipochrome in the perivascular spaces and within neurons are reported changes associated with senile atrophy in domestic animals. Observed changes appeared localised and mild and were probably not significant clinically (Cantile 2016).

Thyroid follicular cysts and adenomatous hyperplasia have been described in both arctic (Hudson Bay) and St. Lawrence beluga populations. Cysts did not appear to be correlated with age and did not appear to have a clinical implication nor a clear suspected etiology, while the volume of adenomatous hyperplasia was positively correlated with age (Mikaelian 2003).

The scarce presence of spermatozooids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989). Focal accumulation of testicular lymphocytes is usually an incidental finding in most species (Foster 2015).

The signification of the muscular lesions observed is uncertain. Changes observed in the muscle could be artifactual and cannot be distinguished from hyperacute muscle injury without fixation of the muscle tissue in a stretched position. (Cooper 2016).

Please note that lesions deemed associated with the harvesting method are not described in this report.

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DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Émilie L. Couture, Hendrickson Island, 2015-07-26.

Sex: Male

Age: 31

Weight: ND

Body score (subjective): Excellent (bulging epaxial muscles)

Length: 437 cm

Carcass condition score: 2

External examination

An adult white male with markedly curved flippers is examined. A harpoon is located to the right of the dorsal crest. Gunshot wounds are located on the dorsal left thoracic and dorsal neck region.

Internal examination

Thyroid: Five cystic structures of 0.5 to 1cm in diameter were noted within the thyroid parenchyma. A nodule, approximately 7 mm in size is also noted within the parenchyma (pictures taken).

Lungs: Occasional nematodes, black and white in coloration and 1x 70 mm in size are found clustered in airways of both lungs. Occasional regions of irregular parenchymal induration of approximately 2-3 cm and beige in color are noted in the lungs.

Lymph nodes: A single round and firm nodule 1 cm in diameter, with a necrotic center is found in a mesenteric lymph node. The mesenteric lymph nodes are of a normal size.

Heart: Irregular, red and smooth irregularities are noted at the junction between the right atrio-ventricular valves and the chordae tendineae (pictures taken).

Stomach and intestines: A single squid beak is noted in the stomach's first compartment. The intestines are distended with bile coloured fluids.

The following structures were without noticeable gross lesions; shoulder joints, muscles, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, liver, lymph nodes (tracheobronchic), urinary system (bladder, kidneys), spleen.

HISTOPATHOLOGY

Heart (B, C): A fibrin clot surrounds the chordae tendinae and does not seem to be adhered to them. Post mortem change.

Muscle (D): Multifocal small to moderate sarcolemmal disruptions are seen. They present as clear spaces of various size within the myocytes, containing acidophilic finely granular material and slightly coarser brown pigments. These spaces are most often seen at the junction between myocytes. Multiple, cells with a prominent nucleus are associated with these lesions. No particularity is noted on PAS coloration.

Thyroid (D, E): Large follicular cysts, from 2 mm in diameter to 5x10 mm are noted on one section (D). Cysts are filled with colloid, and the larger one contains mineralized particles in its center. The smaller cyst is lined by a cuboidal to columnar epithelium that form low papillary projections within the lumen. The larger cyst is surrounded by a fine layer of conjunctive tissue. Flat epithelial cells are sometimes seen in its periphery, while multifocally epithelial nucleus without distinguishable cytoplasm margins are also seen. Two small nodules of hyperplasia, 1 mm in diameter, are noted (E). these nodules are formed of densely packed cords of follicular epithelium where cells are large and polygonal with a foamy cytoplasm. Small amount of colloid may be seen within small follicular structures. In one follicle, multiple follicular structures are filled with desquamated follicular cells.

Central nervous system (S, T, U): Mild perivascular lymphoplasmacytic and histiocytic cuffing associated with light yellow fine granular material (S, T). Similar granular yellow material is seen within neurons.

Reproductive tract (F, I): No sperm is stored in the epididymis (I), and there is no significant spermatogenesis within the seminiferous tubules (F). There is a focal interstitial aggregation of mononuclear inflammatory cells in the testis surrounding degenerated seminiferous tubules. A focal, small (0.15 mm in diameter) cystic structure filled with pale acidophilic liquid is noted in the epididymal epithelium, lined with a flat epithelium.

Lymph nodes (F, H, G, I): Focally, mild infiltration of histiocytes and eosinophils is seen within a lymphonodular capsule (I). A single nodule with a thick fibrous wall and degenerate cells within its center is noted (H). Two trematode egg capsules are noted within this necrotic center, as well as long strands of collagenous material and granular, golden pigments. No microorganisms are noted on Zielh-Nielsen and Brown&Hopps coloration (H).

Lungs (M to R +K, L): Multifocal mild to marked interstitial inflammatory cells (histiocytes, eosinophils) infiltration is noted and may affect various parts of the parenchyma, such as alveolar walls, bronchioles, terminal bronchi, perivascular spaces and subcapsular area. Lesions may be associated with multinucleated giant cells, with cut sections of degenerated nematodes approximately 0.1mm in diameter (M-s). Some of these areas, usually more extensive, are associated with the intraluminal presence of eosinophils, alveolar macrophages and erythrocytes. In some of the most affected sections, airways may also be filled with a proteinaceous liquid with some eosinophils and alveolar macrophages or with degenerated cells and neutrophils (O-m). In affected regions with mostly histiocytic infiltrations, lesions are focal and discrete, or relatively diffuse and mild (M-m,p) and may be associated with small amount of proteinaceous material within airways as well as scarce alveolar macrophages and eosinophils. Varying degrees of fibroplasia may be associated with these regions: peribronchiolar architecture may appears modified, (O-m).

On one extra slide provided (K), large proportions of the parenchyma are affected by inflammatory infiltrations listed above. Cut sections of a 0.5 mm in diameter nematode with a thick pigmented and serrated cuticle are located in a terminal bronchi. While moderate inflammatory cell infiltration is associated with the submucosa and periphery of this airway and there is mild acidophilic liquid and some inflammatory cells within the lumen, a massive inflammatory cell infiltration (histiocytes, eosinophiles) surrounds the associated bronchiole and extensively obliterates alveoles. Bronchiolar lumen is filled with neutrophils, degenerated cells, and bacterial colonies and adjacent alveoles are filled with proteinaceous material. On L, sections display similar but milder lesions. Similar sections of nematodes are seen in a larger airway and are associated with a small amount of proteinaceous material as well as cellular debris and inflammatory cells.

No significant lesions on the examined lesions from: Skin and blubber (A), bladder (A), spleen (D), liver (E), pancreas (J), kidney (J), duodenum (K), pituitary (T), adrenals (V to AA)

Histomorphologic diagnostics

1. Multifocal thyroid follicular cysts and nodular hyperplasia
2. Mild cerebral lipofuscinosis
3. Inactive spermatogenesis
4. Focal lymphoplasmacytic orchitis
5. Focal lymphonodular encapsulated trematodiasis

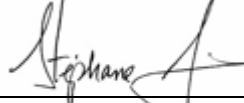
6. Multifocal verminous suppurative and eosinophilic bronchopneumonia (*Halocercus* sp., *Stenurus* sp.)

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2016-02
PATHOLOGY NO.: P3467-16

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult female beluga whale was harvested off on 2016-07-08. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (probable)**
2. **Gestation – first trimester**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed fair based on limited available pictures and morphometric measurements. No weight was obtained in this animal, circumferences are similar to other females and lumbar epaxial muscles do not appear sunken. The size of the foetus indicates that this female was bred the same year and absence of lactation indicates she did not have a suckling veal.

The lung lesions observed in this individual were mild and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal. The predominant mononuclear cells in conjunction with some eosinophils may indicate a chronic parasitic infection. Lungworms, mainly *Halocercus monoceris*, *H. taurica* and *Stenurus arctomarinus*, are the most frequent cause of parasitic pneumonia in Saint-Lawrence beluga whales (Lair 2016).

The lymph node lesions were mild and likely non-significant. Focal cortical reactions may be associated with hematogenous drainage of pathogenic organism (bacterial, parasitic) through the lymph node (Valli 2015).

References

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DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Émilie L. Couture, Dr. Rozenn Le Net, Jasmine Brewster, Shannon MacPhee, John Noksana Sr, Hendrickson Island 2016-07-08.

Sex: Female

Age: pending

Weight: ND

Epaxial muscle weight (L1-L12): ND

Body score (subjective): good

Length: 342 cm

Carcass condition score: 2

External examination

An adult female, white in coloration is examined. Multifocal, slightly depressed circular lesions with an irregular surface are noted mainly on the dorsal face. A few hundreds of those lesions were seen and their size ranged between 1 and 3 cm in diameter.

Internal examination

Reproductive system (cervix, uterus and ovaries): A large amount of thick mucus is seen in the cervical lumen. A moderate distension of both uterine horns is noted. A more pronounced distension is noted on the right side, in association with a dead male foetus (length: 18.5 cm, weight: 141 g). The surface of the right ovary is irregular, with a 4.5 cm *corpus luteus*. No abnormality is noted.

Mammary gland: No milk is extracted when the mammary glands are pressed.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are slightly distended with bile coloured fluids.

The following structures were without noticeable lesions: Shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, lungs, heart, liver, spleen, lymph nodes (tracheobronchic, mediastinal, mesenteric), urinary system (bladder, kidney).

HISTOPATHOLOGY

Lymph nodes (B, K): On two lymph node sections (B,K), focal accumulations of neutrophils and or eosinophils are located at the center of follicles located in the outer cortex. A marked infiltration of eosinophils is seen in the conjunctive tissue adjacent to this lymph node. A similar, but milder infiltration is seen in the conjunctive tissue of another section.

Lungs (C to H): On eleven of the observed sections, there is a mild to moderate, multifocal lymphoplasmacytic interstitial infiltration rarely associated with eosinophiles and macrophages accumulation within alveoli. For all sections, these changes are mild and limited to a few focal airways never representing more than 5% of the section's surface.

Mammary gland (L): inactive glandular tissue.

No significant lesions on the examined sections from: Heart (A), muscle (A), pituitary (B, R), lymph nodes (B), Spleen (I, L), liver (I), pancreas (I), thyroid (J), kidneys (J), bladder (K), duodenum (L), Ovary (M, N) Central nervous system (P, Q, R), adrenal glands (S to X)

Histomorphologic diagnostics

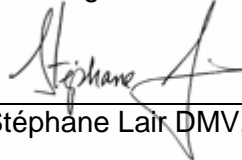
- Mild multifocal interstitial pneumonia and eosinophilic alveolitis
- Mild multifocal cortical suppurative or eosinophilic lymphadenitis
- Inactive mammary gland

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2016-03
PATHOLOGY NO.: P3468-16

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested off of on 2016-07-08. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Stenurus* sp., *Halocercus* sp. possible)**
2. **Renal nematodiasis (*Crassicauda giliakiana*)**
3. **Chronic oesophageal ulcers (possible herpesvirus infection)**
4. **Inactive spermatogenesis**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed good based on limited available pictures and morphometric measurements (epaxial muscle weight).

The morphology of the parasite observed on histologic sections correspond to a larval form of *Stenurus* sp. The presence of this parasite was associated with lesions and could explain the lesions noted in other sections, although the undetected presence of *Halocercus* sp., another pseudaliid nematode, cannot be excluded and has been described with similar lesions. Alveolar hemorrhages associated with inflammatory reactions are indicative of an acute infection, while inflammatory reactions surrounding fibrous parenchyma and foci of mineralization are indicative of the chronic parasitic presence. Despite these observations, the lung lesions in this individual were mild and did not affect its health considering

the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal (Lair 2016, Measures 2018).

The location and morphology of the encapsulated renal nematode is typical of the genus *Crassicauda* sp, a spirurid nematode found in mysticetes and odontocetes. *Crassicauda giliakiana* is generally site specific and associated to the urogenital system (Measures 2018). Although significant lesions can sometimes be associated with this parasite, in this animal the small proportion of renal tissue affected did not negatively impact its health.

The etiology behind the oesophageal ulcers is uncertain and there is no frozen tissue available to consider molecular diagnostic testing. One possible differential for this presentation, despite no clear inclusion bodies found within the lesions would be a herpesvirus infection. Herpesviruses commonly circulate in odontocetes, including St. Lawrence beluga whales. This virus generally becomes latent following an initial infection and recrudescence is associated with physiologic stress and/or immunosuppression. Characterized odontocete herpesviruses are considered to be cetacean-specific and to have co-evolved with their hosts. They are not known to be zoonotic. Viruses have been isolated both from healthy tissues or from lesions such as encephalitis, acute necrotizing lesions, genital ulcers and dermatitis (alphaherpesvirus) and oral lesions, genital ulcers, plaques or proliferating lesions (gammaherpesviruses) (Duignan, 2018).

The scarce presence of spermatozooids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

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- Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. Veterinary Pathology. 2016 Jan;53(1):22-36.
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DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Émilie L. Couture, Dr. Rozenn Le Net, Jasmine Brewster, Shannon MacPhee, John Noksana Sr. on Hendrickson Island on the 2016-07-08.

Sex: Male

Age: ND
Weight: ND
Epaxial muscle weight (L1-L12): 28 kg
Body score (subjective): good
Length: 404 cm (13 ft 3 in.)
Carcass condition score: 2

External examination

An adult male, white in coloration is examined. Multifocal, slightly depressed circular regions with an irregular surface are noted multifocally. Less than 100 of those lesions were seen and their size ranged between 1 and 3 cm in diameter.

Internal examination

Lungs: In the median part of the left lung, a small circular, but irregular, induration of the parenchyma of 1 cm in diameter is noted. This region is dark red in color and not well defined.

Kidney: A round cystic mass of 4 cm by 3 cm with a thick, white capsule is noted at the cranio-lateral surface of the left kidney. It contains clear, slightly orange fluid as well as a single long filamentous structure of approximately 15 cm.

Oesophagus: Approximately 100 slightly depressed circular lesions are noted on the mucosal surface, mostly in the cranial part of the oesophagus. Their size ranges from 0.3 to 2 cm in diameter. Their surface is slightly irregular and at the center of many of them, a deep ulcer of 1 to 3 mm of diameter is noted.

Stomach and intestines: No food material is noted in the digestive tract, but the stomach and the first portion of intestines contains a very large amount of slightly brown and green liquid (possibly seawater). The intestines are slightly distended with bile coloured fluids.

The following structures were without noticeable lesions: shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, heart, liver, spleen, lymph nodes (tracheobronchic, mediastinal, mesenteric), urinary bladder, reproductive system (testes and epididymis).

HISTOPATHOLOGY

Lymph nodes (B, O): Good cellularity, some germinal centers discernible and are especially prominent in one section (B). In another section, a diffuse and irregular section of fibrosis is noted.

Lungs (C to H): On all of the observed sections, there is a mild to moderate, multifocal lymphoplasmacytic interstitial infiltration rarely associated with eosinophilic and histiocytic cell accumulation within alveoli and bronchioles. For most sections (15/18), these changes are mild and limited to a few focal airways representing less than 5% of the section's surface. Focal fibroplasia with histiocytic, lymphocytic and eosinophilic interstitial infiltration is associated with a foci of mineralization and small amounts of granular eosinophilic material (E-m). Focal region of alveoli overinflation may be associated with proteinaceous material within alveoli and adjoining airways mild cellular infiltration (F-s). In the intermediate lung section of the median portion of the right lung lobe, lesions encompass more than 30% of the section and include inflammatory infiltration (mononuclear, eosinophilic) of the interstitial tissue (including marked infiltration surrounding an artery) as well as alveolar distention with blood, proteinaceous liquid and inflammatory cells (alveolar macrophages, eosinophils and neutrophils). Within an affected region, a small nematode (0.15 mm diameter) is noted in cut section at the intersection between a bronchiole and alveoli (G-m). A focally extensive, linear region of fibrosis with histiocytic and eosinophilic infiltration between and extending beyond two thick walled arteries is noted in the deeper section of the caudal right lung (H).

Kidney (J, K, L): The kidney parenchyma is normal for both sides. The cystic mass on the left kidney (K, L as per gross examination) is composed of a thick accumulation of fibrous fibers associated with a local compression of the renal parenchyma (L). Multifocally extensive infiltration of inflammatory cells are noted in the compressed parenchyma and capsule margin. Inflammatory infiltrates are predominantly composed of mononuclear cells (mostly macrophages, including multinucleated giant cells) and eosinophils. Multiple cut sections of a nematode with a thick, serrated cuticle are visible (K, L).

Oesophagus (M – 4 different cuts): Diffusively, there is a mild lymphoplasmacytic and histiocytic (rarely, polymorphonuclear cells) infiltration of the superficial dermis and dermal papillae. The stratified squamous epithelium thickness is irregular thorough the section. In the thinnest portion, there is a focal ulceration of the keratin layer. In this specific region as well as multifocally on the sections and especially within the stratum germinativum, keratinocyte arrangement is disorganized and associated with vacuolation of keratinocytes, chromatin margination (sometimes associated with a clear, glassy appearance structure in the nucleus). Seldom, mitosis figures are noted within the stratum germinativum.

Reproductive tract (N, O): mostly inactive sperm production in the testis (N), empty epididymis (O).

No significant lesions on the examined sections from: skin and blubber (A), heart (A), Muscle (A), pancreas (A), spleen (I), liver (I), thyroid (J), kidneys (J), duodenum (M,N), central nervous system (P,Q,R), hypophysis (R), adrenal glands (S to X)

Histomorphologic diagnostics

- Lymphonodular fibrosis
- Focal, chronic renal nematodiasis (*Crassicauda giliakiana*)
- Mild, chronic active eosinophilic and histiocytic verminous bronchopneumonia (*Stenurus* sp., possibl *Halocercus* sp)
- Oesophageal ulcers, possible herpesvirus infection
- Inactive spermatogenesis

Resident:

Émilie L. Couture, DMV, IPSAV, DES

Pathologist:

Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2016-04
PATHOLOGY NO.: P3470-16

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested off of on 2016-07-09. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Mild eosinophilic pneumonia (lungworms suspected)**
2. **Renal nematodiasis (*Crassicauda giliakiana*)**
3. **Lymph node trematodiasis**
4. **Digestive nematodiasis (*Anisakidae*)**
5. **Adrenocortical cysts**
6. **Inactive spermatogenesis**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was not determined due to the lack of pictures and weights.

Lung lesions observed in this individual were mild and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal. The predominant mononuclear cells in conjunction with some eosinophils may indicate a chronic parasitic infection. Lungworms, mainly *Halocercus monoceris*, *H. taurica* and *Stenurus arctomarinus*, are the most frequent cause of parasitic pneumonia in Saint-Lawrence beluga whales (Lair 2016).

The location and morphology of the encapsulated renal nematode is typical of the genus *Crassicauda* sp, a spirurid nematode found in mysticetes and odontocetes. *Crassicauda giliakiana* is generally site specific and associated to the urogenital system (Measures 2018). Although significant lesions can sometimes be associated with this parasite, in this animal the small proportion of renal tissue affected did not negatively impact its health.

Trematodes have not been reported in beluga lymph node. In Hector's dolphins (*Cephalorhynchus hectori*), an adult trematode had a morphology corresponding to *Campula* spp and was noted to be encapsulated in the mesenteric lymph nodes, along with similarly encapsulated aggregates of trematode eggs associated with a strong macrophagic and fibrosing reaction (Hutton 1987). In general, digenean trematode of the genus *Campula* are associated with the liver, bile and pancreatic apparatus in odontocetes. They may cause variable level of lesions and their life cycle is poorly known. In this case, the encapsulated eggs in the lymph nodes were unlikely to have a negative health effect (Measures 2018).

Although precise morphology of the gastric nematode is lacking, nematodes from the family Anisakidae are found in cetacean stomach. *Anisakis simplex* is the most prevalent gastric helminth in St. Lawrence beluga whales and is usually found in the first compartment of the stomach, while *Contracaecum* sp. were the most prevalent and abundant in beluga whales sampled from the Mackenzie River Delta (Lair 2016, Measures 1995, Wazura 1986).

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes (Lair, 1997).

Changes observed in the muscle could be artifactual and cannot be distinguished from hyperacute muscle injury without fixation of the muscle tissue in a stretched position. (Cooper 2016)

The scarce presence of spermatozoids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

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- Wazura KW, Strong JT, Glenn CL, Bush AO. Helminths of the beluga whale (*Delphinapterus leucas*) from the Mackenzie River Delta, Northwest Territories. *Journal of Wildlife Diseases*. 1986 Jul;22(3):440-2.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Émilie L. Couture, Dr. Rozenn Le Net, Jasmine Brewster, Shannon MacPhee, John Noksana Sr. on Hendrickson Island on the 2016-07-09.

Sex: Male

Age: ND

Weight: ND

Epaxial muscle weight (L1-L12): ND

Body score (subjective): ND

Length: 434 cm

Carcass condition score: 2

External examination

An adult male, white in coloration is examined. Very few multifocal, slightly depressed circular lesions with an irregular surface are noted on the dorsum. Their size ranged between 1 and 2 cm in diameter.

Internal examination

Kidney: A round 1.5 cm in diameter nodule is present on the dorsal aspect of the one of the kidney. It is well defined, has a very hard consistency and its shape is irregular (circumvolute)

Mesenteric lymph node: Approximately ten well defined nodular masses are noted within the mesenteric lymph nodes. They are very hard, well delimited, regular, slightly yellow with a size ranging from 1 to 2.5 cm in diameter. On cut section, the centre of the structure is thick and dry, green-brown in coloration: it appears to be necrotic.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are slightly distended with bile coloured fluids.

The following structures were without noticeable lesions: Shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, lungs, heart, liver, spleen, lymph nodes (tracheobronchic, mediastinal, mesenteric), urinary bladder, reproductive system (testes and epididymis), esophagus.

HISTOPATHOLOGY

Muscle (B): Approximately a quarter of the muscle fibers appear enlarged and present a certain loss in the demarcation of their striations, possibly because of proteinaceous material between the striations. These fibers are generally surrounded by a clear halo sometimes filled with proteinaceous material.

Lungs (C-H): Multifocal, mild mononuclear (rarely, with eosinophils) interstitial inflammatory infiltrates, rarely associated with intraluminal alveolar and bronchiolar accumulations of macrophages and eosinophils.

Lymph nodes (I, J, K, Y, Z, AA): In all sections (I, J, K), there are diffuse, moderate to marked areas of fibrosis within various zones of the parenchyma, including in the center of lymphoid aggregations. Multifocal aggregations of eosinophils are noted within germinal centers of the outer cortex in some sections, as well as multifocal aggregation of granular to ovoid eosinophilic material is surrounded by macrophages and multinucleated giant cells in different location of the lymph nodes (e.g. paracortex, cortical germinal center) (I, J). The nodular masses noted grossly consist of a thick fibrous capsule with degenerate inflammatory cells in the lumen and rare triangular shaped, thick pigmented shelled trematode eggs (AA). A large amount of eggs sharing the same morphology is also seen in a distinct non encapsulated cluster adjacent to a fibrosed nodule (AA). Macrophagic cells surround this aggregate, often infiltrating the lumen of the eggs located in it's periphery.

Reproductive system (K, L): No sperm is stored in the epididymis (K), and there is not significant spermatogenesis within the testis (L)

Parasite (L): A longitudinal section of a nematode is presented which appears to contain a large number of thin walled eggs. And has a moderately thick, yellow serrated cuticle. (Anisakidae)

Kidneys (M, N): The renal parenchyma is generally normal. On one section (N), there is an extension region of nodular fibrosis encompassing several subsections of circumferential fibrous aggregation surrounding sections of a nematode with a thick, serrated cuticle. Peripherally to these fibrous capsules, there is a few, multifocal aggregation of mononuclear cells (principally macrophages) and more rarely, eosinophils.

Adrenal glands (S to X): Rare (4) microscopic cysts of approximately 0.4 mm in diameter, clustered close together (V). These cysts are delimited by a thin strand of conjunctive tissue and are associated with minimal vacuolar degeneration of adjacent adrenocortical cells

No significant lesions on the examined sections from: heart (A), skin and blubber (A), muscle (B), central nervous system (P, Q, R), thyroid (B), spleen (B) bladder (J), pancreas (K), liver (L), duodenum (O)

Histomorphologic diagnostics

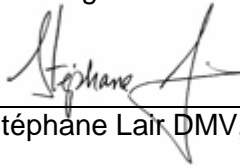
- Focal, encapsulated chronic renal nematodiasis (*Crassicauda giliakiana*)
- Mild multifocal interstitial pneumonia and eosinophilic alveolitis
- Mild multifocal cortical eosinophil aggregation within lymph nodes
- Multifocal lymph node trematode egg encapsulation
- Multifocally extensive, moderate to marked lymph node fibrosis
- Rare adrenocortical cyst formation
- Inactive spermatogenesis
- Digestive nematodiasis (Anisakidae)

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2016-05
PATHOLOGY NO.: P3471-16

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested off of on 2016-07-10. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Stenurus* sp., possibly *Halocercus* sp.)**
2. **Renal nematodiasis (*Crassicauda giliakiana*)**
3. **Lymph node trematodiasis**
4. **Thyroid - follicular cysts and nodular hyperplasia**
5. **Molt**
6. **Mild cholangiolitis**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed excellent based on available pictures and morphometric measurements. This animal was the heaviest one weighed and one of the longest. Body mass: total length ratio was one of the highest with 2.58. Epaxial musculature and blubber appear well rounded in the lumbar area.

The lung lesions observed in this individual were mild and did not affect its health considering the mild gross lesions observed, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal. The nematodes observed in the larger airways of this individual have a morphology that correspond to the pseudaliid nematode *Stenurus* sp. Pathogeny associated with these lungworms is typically associated to airway occlusion due to the physical presence

of parasite within airways. Although its role in bronchopneumonia lesions cannot be excluded, observations also correspond to typical lesions associated with pseudaliid nematodes of the genus *Halocercus* that may not have been present on examined sections other than in a degenerate, mineralized form. *Halocercus monoceris* and *H. taurica* are reported in Saint-Lawrence beluga whales (Lair 2016).

The location and morphology of the encapsulated renal nematode is typical of the genus *Crassicauda* sp, a spirurid nematode found in mysticetes and odontocetes. *Crassicauda giliakiana* is generally site specific and associated to the urogenital system (Measures 2018). Although significant lesions can sometimes be associated with this parasite, in this animal the small proportion of renal tissue affected did not negatively impact its health.

Trematodes have not been reported in beluga lymph node. In Hector's dolphins (*Cephalorhynchus hectori*), an adult trematode had a morphology corresponding to *Campula* spp and was noted to be encapsulated in the mesenteric lymph nodes, along with similarly encapsulated aggregates of trematode eggs associated with a strong macrophagic and fibrosing reaction (Hutton 1987). In general, digenean trematode of the genus *Campula* are associated with the liver, bile and pancreatic apparatus in odontocetes. They may cause variable level of lesions and their life cycle is poorly known. In this case, the encapsulated eggs in the lymph nodes were unlikely to have a negative health effect (Measures 2018).

The histology of the skin lesions observed in this animal are consistent with a normal molt process. Similar changes were noted in the spring migration as well as in the summer estuarine passage of Hudson Bay beluga whales. The dorsal localisation of these lesions suggests may be consequent with recent passage and skin abrasion under ice sections (St. Aubin 1990).

Follicular cysts and adenomatous hyperplasia have been described in both arctic (Hudson Bay) and St. Lawrence beluga populations. Cysts did not appear to be correlated with age and did not appear to have a clinical implication nor a clear suspected etiology, while the volume of adenomatous hyperplasia was positively correlated with age (Mikaelian 2003). In this case, nodular hyperplasia was not noted on the examined section, but were described on gross examination.

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

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- Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. Veterinary Pathology. 2016 Jan;53(1):22-36.
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- Mikaelian I, Labelle P, Kopal M, De Guise S, Martineau D. Adenomatous hyperplasia of the thyroid gland in beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary and Hudson Bay, Quebec, Canada. Veterinary Pathology. 2003 Nov;40(6):698-703.
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DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Émilie L. Couture, Dr. Rozenn Le Net, Hendrickson Island, 2016-07-10.

Sex: Male

Age: ND

Weight: 1147 kg

Epaxial muscle weight (L1-L12): ND

Body score (subjective): excellent

Length: 445 cm

Carcass condition score: 2

External examination

An adult male, white in coloration is examined. Multifocal, slightly depressed circular lesions with an irregular surface are noted mainly on the dorsal face. Less than 100 of those lesions were seen and their size ranged between 1 and 2 cm in diameter.

Internal examination

Thyroid: Five to ten white and firm nodules, 2-3 mm in diameter are present within the parenchyma.

Lungs: At the dorsal surface of the right lung, there are approximately 10 small, white, circular indurations of a few millimeters in diameter. In the median part of the right lung, a small circular, but irregular, mild parenchymal induration of 1 cm in diameter is noted. This region is of a dark red coloration and is not well delimited. Occasional black and white nematodes measuring approximately 0.5 x 100 mm are noted in the small airways of the right lung.

Kidney: Round cystic nodules between 1.5 and 2.5 cm in diameter are noted on the surface of the ventral aspect of the right kidney (n=2) and of the left kidney (n=1). On cut section, they have a thick white capsule and contain clear, slightly sero-sanguineous fluid as well as a long filamentous and gelatinous structure.

Mesenteric lymph nodes: Approximately 5 white nodules with a bony consistency are noted within the mesenteric lymph nodes. On cut section, they contain a necrotic material in the center.

Stomach and intestines: No food material is noted in the digestive tract. The intestines are slightly distended with bile coloured fluids.

The following structures were without noticeable lesions: shoulder joints, muscles, thyroid gland, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, heart, liver, spleen, lymph nodes (tracheobronchic, mediastinal), urinary bladder, reproductive system (testes and epididymis), esophagus.

HISTOPATHOLOGY

Skin and blubber (A): Two sections of skin are assessed. On one, the most superficial portion (stratum externum) is partially torn from the underlying cells, with degenerate epidermal cells as well as granular debris, possibly including bacteria and unicellular organisms. On this section, the most superficial portion of the exposed stratum intermedium presents with cells that have a flatter appearance when compared with cells of same level under a section where the stratum externum / parakeratotic layer is still attached. In the cleavage zone, there is a thin layer of cells that appear swollen when compared with the overlying

sloughing stratum externum and the underlying thinner stratum intermedium. This distinction in cell morphology is not apparent on the rest of the section or on the intact skin section where the stratum externum is intact.

Thyroid (B): There is a marked difference in follicle size through both sections assessed. Multiple large follicles reach 1 to 2 mm in diameter and are generally lined with a cuboidal epithelium. A larger, cystic lesion is composed of several smaller cysts (3 to 8 mm in diameter) surrounded by a thin fibrous capsule and generally lined with a flat epithelium. These cysts appear to contain the same proteinaceous material as in surrounding follicles.

Lungs (D-I and J): There is multifocal, mild to moderate interstitial inflammatory cells (mononuclear and sometimes eosinophils) infiltration of the parenchyma, often in the periphery of bronchioles. Inflammatory cell aggregation may surround granular proteinaceous material or foci of mineralization within the parenchyma (F-p, H-p). Focally, an arteriolar wall, lumen and periphery are also implicated (E-p). Focal accumulation of alveolar proteinaceous material, macrophages and eosinophils may be present. Fibroplasia associated with mononuclear and eosinophilic cell infiltration is rarely noted (D-s, E-p). Multifocal aggregations of alveoli, generally adjoining areas of focal inflammatory infiltrates appear distended with a finely granular material and pearls of proteinaceous material with alveolar macrophages. In the additional sections provided of the superficial right lung induration (J), a nodular fibrous capsule of 2 mm in diameter surrounds a mineralized center. Mononuclear cell infiltrates surround the nodule. Amorphous material, part eosinophilic and part basophilic with regions of mineralisation are also noted in rare bronchioles and terminal bronchi. On another section provided, interstitial inflammatory changes and alveolar accumulation of proteinaceous liquid and inflammatory changes is more locally extensive but is still limited to 15% of the section.

Lymph nodes (K, L, M): There is a focal aggregation of neutrophils or eosinophils in cortical aggregation of lymphocytes (K, M). A 1 cm in diameter nodule is composed of a moderately thick fibrous capsule and contains a multitude of triangular shaped, lightly colored eggs. These eggs are embedded in fibrous tissue. The shell is often breached, and egg content often appears to be fibrous tissue and/or finely granular mineral material. Several blood vessels are noted within the nodule and are sometimes associated with focal mononuclear cell infiltration.

Reproductive tract (L, N): No sperm is stored in the epididymis (N), and there is not significant spermatogenesis within the testis (L)

Thymus (N): The gross organisation of this tissue is reminiscent of the pancreatic architecture. However, upon closer examination, it appears to be constituted exclusively of mononuclear cells, along with multifocal structures resembling lymph node germinal centers with dense acidophilic aggregates and laminated cells in their center (Hassall's corpuscles). A large proportion of lymphoid cells contain dark refractile pigments within their nucleus

Spleen (O): There is multifocal aggregation of brown pigmented material in splenic macrophages.

Kidneys (P, Q): On both sections observed (P), a thick-walled fibrous capsule focally and extensively obliterates the renal parenchyma. Within the lumen of the fibrous cyst, proteinaceous material as well as cut sections of a large (approximately 5 mm in diameter) nematode with a thick, sleek and pigmented cuticle. Renal parenchyma is otherwise normal (P, Q)

No significant lesions on the examined sections from: heart (C), muscle (C, K), central nervous system (R, S, T), adrenal glands (U to Z), pancreas (L), Liver (O), stomach (O), pituitary gland (R)

Histomorphologic diagnostics

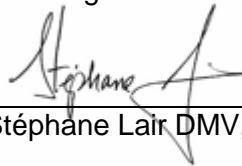
- Active shedding of the skin stratum externum
- Thyroid follicular cysts
- Chronic-active mild multifocal eosinophilic bronchopneumonia and nodular mineralisation (*Halocercus* sp. likely)
- Multifocal lymph node trematode egg encapsulation
- Multifocal, encapsulated chronic renal nematodiasis (*Crassicauda giliakiana*)

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



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ARHI-DL-2016-06

PATHOLOGY NO.: P3472-16

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested off of on 2016-07-13. It was hunted around 1:15 pm. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. Parasitic bronchopneumonia (*Halocercus* sp.)
2. Thyroid- follicular cysts
3. Pituitary cysts and psammoma bodies
4. Adrenocortical cysts
5. Inactive spermatogenesis
6. Pancreatic exocrine hyperplasia
7. Lymph node fibrosis
8. Gastric nematodiasis

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed good based on available pictures and morphometric measurements (epaxial muscle weight).

The morphology of the parasite observed in the lungs as well as observed lesions are typical of an infection with a pseudaliid nematode of the genus *Halocercus* (*H. monoceris* has been reported in monodontidae). Alveolar hemorrhages associated with inflammatory reactions are indicative of an active

infection. Despite the fact that some sections appeared the lung lesions in this individual were more affected than in other individuals, there was no associated gross lesion and this likely did not affect its health considering and the good general condition of the animal (Lair 2016, Measures 2018).

Thyroid follicular cysts have been described in both arctic (Hudson Bay) and St. Lawrence beluga populations. They did not appear to be correlated with age and did not appear to have a clinical implication nor a clear suspected etiology (Mikaelian 2003).

Pituitary cysts and psammoma bodies have been described in cetacean species (Cowan 2008) and this observation is not considered to be significant.

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes (Lair, 1997).

The scarce presence of spermatozooids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

An increased deposition of connective tissue in human lymph nodes is reported as one of various possible degenerative changes associated with senescence and appears mostly noted in superficial inguinal nodes (Ahmadi 2013).

Although morphology of the gastric nematode is lacking, nematodes from the family Anisakidae are found in cetacean stomach. *Anisakis simplex* is the most prevalent gastric helminth in St. Lawrence beluga whales and is usually found in the first compartment of the stomach, while *Contracaecum* sp. were the most prevalent and abundant in beluga whales sampled from the Mackenzie River Delta (Lair 2016, Measures 1995, Wazura 1986).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

- Ahmadi O, McCall JL, Stringer MD. Does senescence affect lymph node number and morphology? A systematic review. ANZ Journal of Surgery. 2013 Sep;83(9):612-8.
- Cowan DF, Haubold EM, Tajima Y. Histological, immunohistochemical and pathological features of the pituitary gland of odontocete cetaceans from the western Gulf of Mexico. Journal of Comparative Pathology. 2008 Aug 1;139(2-3):67-80.
- Lair S, Beland P, De Guise S, Martineau D. Adrenal hyperplastic and degenerative changes in beluga whales. Journal of Wildlife Diseases. 1997 Jul;33(3):430-7.
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- Wazura KW, Strong JT, Glenn CL, Bush AO. Helminths of the beluga whale (*Delphinapterus leucas*) from the Mackenzie River Delta, Northwest Territories. Journal of Wildlife Diseases. 1986 Jul;22(3):440-2.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Émilie L. Couture, Dr. Rozenn Le Net, Hendrickson Island on the 2016-07-13.

Sex: Male

Age:

Weight: ND

Epaxial muscle weight (L1-L12): 25 kg

Body score (subjective): good

Length: 424 cm

Carcass condition score: 2

External examination

An adult male, white in coloration is examined. Multifocal, slightly depressed circular lesions with an irregular surface are noted mainly on the dorsal face. Approximately 50 of those lesions were seen and their size ranged between 1 and 2 cm in diameter. Large zone of yellow skin and molting skin are also noticed.

Internal examination

Thyroid: Approximately ten cystic lesions are present within the parenchyma. They are clear to black in colour and firm. Some are simple nodules of 5 mm of diameter whereas others are multi-lobular structures of 2.5 x 1.5 cm.

Stomach and intestines: No food material is noted in the digestive tract. Several nematodes and found in the first compartment of the stomach. Their diameter ranges from 2 to 4 mm and their length range from 7 to 12 cm.

The following structures were without noticeable lesions: shoulder joints, muscles, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, lungs, heart, liver, pancreas, spleen, lymph nodes (tracheobronchic, mediastinal, mesenteric), urinary system (kidneys and bladder), reproductive system (testes and epididymis), esophagus.

HISTOPATHOLOGY

Thyroid (C, D, E): Multiple large adjoining cysts reach 5 to 10 mm in diameter and are generally lined with a flat epithelium (C, D). They are surrounded by a thin fibrous capsule where rare, mildly compressed follicular structures may still be noted. These cysts appear to contain the same proteinaceous material (colloid) as in surrounding follicles.

Lungs (F to K): There is mild to moderate interstitial, notably surrounding arterioles and sometimes bronchioles, infiltration of eosinophils (predominantly) and macrophages. Eosinophils may also be present in the arteriolar vascular wall (G-s, K-m). Multiple surrounding alveoli or corresponding bronchioles are filled with eosinophils and alveolar macrophages, more rarely with erythrocytes (J-m). These changes affect between 5 and 40% of the observed sections and seem more important in the cranial and median sections of the lungs (5-40%) than in the caudal portion (5%). Focally, clusters of macrophages including giant multinucleated cells surround granular eosinophilic material (F-s). Focally, cut sections of a nematode of approximately 0.1 mm in diameter are seen within a small pulmonary arteriole (G-m)

Lymph nodes (L, M): On two of the four sections observed (L), particularly, there is a general fibrosis of the parenchyma, sometimes associated with the center or cortical lymphocyte aggregations. On one section (M), The margin between regions of dense fibrosis and lymphoid centers contains marked amounts of eosinophils.

Reproductive tract (M, N): No sperm is stored in the epididymis (M), and there is no significant spermatogenesis within the testis (N)

Liver (N): Diffusely, there is mild amounts brown pigmented granules in the lumen of hepatocytes.

Hypophysis (Q): Multifocally and sometimes closely aggregated, small punctual foci of mineralisation (Psammoma bodies) are noted in the adenoypophysis. A single foci of nodular chromophobe cell aggregation is noted and seems associated with the presence of multiple clear vacuoles. Two small cysts of approximately 1 mm in diameter are also noted.

Adrenal (T to Y): Approximately five cystic structures, usually single are noted and range from 0.52 to 1 mm in size (W X). They are surrounded by a thin endothelium.

No significant lesions on the examined sections from: skin and blubber (A), muscle (A), pancreas (A), heart (B), bladder (B), kidneys (O), spleen (N), duodenum (P), central nervous system (Q, R, S)

Histomorphologic diagnostics:

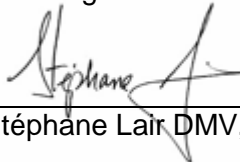
- Thyroid cysts
- Extensive lymph node fibrosis and eosinophilic infiltration
- Mild to moderate eosinophilic bronchopneumonia and focal intravascular nematodiasis (*Halocercus* sp.).
- Extensive lymph node fibrosis and eosinophilic infiltration
- Inactive spermatogenesis
- Psammoma bodies and cysts within adenoypophysis
- Mild, sparse small adrenal cysts formation

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

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 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2016-07
PATHOLOGY NO.: P3473-16

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested off of on 2016-07-13. It was hunted around 1:30 pm. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Halocercus* sp., *Stenurus* sp.)**
2. **Thyroid - follicular cyst**
3. **Pituitary gland cysts**
4. **Adrenocortical cysts**
5. **Inactive spermatogenesis**
6. **Lymph node fibrosis**
7. **Gastric nematodiasis**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed excellent based on available pictures and morphometric measurements. This animal was the second heaviest one weighed. Body mass: total length ratio was one of the highest with 2.57. Epaxial musculature and blubber appear well rounded in the lumbar area, while the thoracic area appears well rounded.

The morphology of the parasite observed in the histopathologic examination of the lungs as well as associated lesions are typical of an infection with a pseudaliid nematode of the genus *Halocercus* (*H.*

monoceris has been reported in monodontidae) The nematodes observed in the larger airways of this individual have a morphology that correspond to the pseudaliid nematode *Stenurus* sp. Pathogeny associated with these lungworms is typically associated to airway occlusion due to the physical presence of parasite within airways. (Lair 2016, Measures 2018). The lung lesions observed in this individual were mild and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal.

Thyroid follicular cysts have been described in both arctic (Hudson Bay) and St. Lawrence beluga populations. They did not appear to be correlated with age and did not appear to have a clinical implication nor a clear suspected etiology (Mikaelian 2003).

Pituitary cysts have been described in cetacean species (Cowan 2008) and this observation is not considered to be significant.

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes (Lair, 1997).

The scarce presence of spermatozooids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

An increased deposition of connective tissue in human lymph nodes is reported as one of various possible degenerative changes associated with senescence and appears mostly noted in superficial inguinal nodes (Ahmadi 2013).

Although morphology of the gastric nematode is lacking, nematodes from the family Anisakidae are found in cetacean stomach. *Anisakis simplex* is the most prevalent gastric helminth in St. Lawrence beluga whales and is usually found in the first compartment of the stomach, while *Contracaecum* sp. were the most prevalent and abundant in beluga whales sampled from the Mackenzie River Delta (Lair 2016, Measures 1995, Wazura 1986).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

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- Lair S, Beland P, De Guise S, Martineau D. Adrenal hyperplastic and degenerative changes in beluga whales. *Journal of Wildlife Diseases*. 1997 Jul;33(3):430-7.
- Lair S, Measures LN, Martineau D. Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, from 1983 to 2012. *Veterinary Pathology*. 2016 Jan;53(1):22-36.
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- Wazura KW, Strong JT, Glenn CL, Bush AO. Helminths of the beluga whale (*Delphinapterus leucas*) from the Mackenzie River Delta, Northwest Territories. Journal of Wildlife Diseases. 1986 Jul;22(3):440-2.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Émilie L. Couture, Dr. Rozenn Le Net, Hendrickson Island, 2016-07-08.

Sex: Male

Age: ND

Weight: 1058 kg

Epaxial muscle weight (L1-L12): 29 kg

Body score (subjective): excellent

Length: 411 cm

Carcass condition score: 2

External examination

An adult male, white in coloration is examined. Multifocal, slightly depressed circular lesions with an irregular surface are noted mainly on the dorsal face. Approximately 50 of those lesions were seen and their size ranged between 1 and 2 cm in diameter.

Internal examination

Thyroid: A cystic structure of 1.5 cm in diameter is noted in the right lobe of the thyroid. It is enveloped by a thin white capsule and contains a viscous fluid, with a mild yellow tinge and opacity and multiple white specks.

Lungs: Occasional black and white nematodes measuring approximately 0.5 mm in diameter and a few centimeters in length are noted in the small airways of both lungs.

Urinary bladder: At the apex of the bladder, surrounding the urachus there are multiple black punctuations of 1 mm in diameter.

Stomach and intestines: No food material is noted in the digestive tract. Several nematodes and found in the first compartment of the stomach. Their diameter ranges from 2 to 4 mm and their length range from 7 to 12 cm. The intestines are moderately distended with bile coloured fluids.

The following structures were without noticeable lesions: shoulder joints, muscles, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, heart, liver, pancreas,

spleen, lymph nodes (tracheobronchic, mediastinal, mesenteric), kidneys, reproductive system (testes and epididymis), esophagus.

HISTOPATHOLOGY

Thyroid (B, C): There appears to be a marked size difference between follicles in both sections observed. Multiple large follicles adjacent to each other reach 1 to 2 mm in diameter and are generally lined with a cuboidal epithelium. Their colloid content is slightly heterogenous, with colloid having a bead appearance and large round cells with a small basophilic nucleus and a foamy cytoplasm multifocally in the follicular lumen. A portion of a cyst is also present. The wall is thick and fibrous, with a cuboidal epithelium. **Lymph nodes (C, J):** On one section, there is moderate, multifocally extensive areas of fibrosis in the parenchyma.

Lungs (D to I): Mild to moderate interstitial infiltration of macrophages and eosinophils are seen in different portion of the parenchyma (alveolar, perivascular and peribronchiolar), as well as intraluminal accumulation macrophages and eosinophils. Within one of the affected sections, cut sections of nematodes (n=2), 0.1 mm in diameter are noted both within the parenchyma and bronchioles (E-p). Similar cut sections (n=3) are also noted within bronchioles not associated with inflammatory infiltration (F-p). Described lesions usually encompass 5% or less of observed sections (11/18), but may affect up to 25% of the section (10-25% in 5/18 sections).

Pancreas (K): multifocally, acinic cells appear swollen, with a condensed nucleus. Vacuoles are often present within the acinar cell cytoplasm.

Reproductive system (K, L): No sperm is stored in the epididymis (K), and there is not significant spermatogenesis within the testis (L)

Bladder (N): On one of both sections evaluated, multifocal distension and congestion of blood vessels are noted in the submucosa.

Pituitary gland (O): A single, 3 mm in diameter cyst is noted on the examined section, lined with a cuboidal to flat epithelium.

Adrenal gland (R to X): Approximately 10 groups of small cysts or single cysts of up to 2 mm in size (W) are noted. Groups of cysts are constituted of multiple small cysts (from smaller than 0.1 mm to 0.3 mm in diameter separated by fine strands of conjunctive tissue (spider web appearance) (T).

No significant lesions on the examined sections from: heart (A, B), muscle (A), skin and blubber (A), liver (L), spleen (L), kidneys (M), stomach (N), central nervous system (O, P, Q)

Histomorphologic diagnostics:

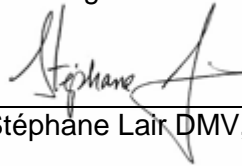
- Focal thyroid follicular cyst
- Lymph node fibrosis
- Mild, multifocal verminous bronchopneumonia (*Halocercus* sp.)
- Multifocal pancreatic hyperplasia
- Inactive spermatogenesis
- Focal pituitary cyst
- multifocal small adrenocortical cysts formation

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2016-08
PATHOLOGY NO.: P3474-16

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested off of on 2016-07-13. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. Parasitic bronchopneumonia (*Halocercus* sp., *Sternurus* sp)
2. Lymphonodular trematodiasis
3. Renal nematodiasis (*Crassicauda giliakiana*)
4. Thyroid - follicular cyst
5. Pituitary cysts
6. Adrenocortical cysts and nodular hyperplasia
7. Inactive spermatogenesis
8. Sinusal nematodiasis
9. Gastric nematodiasis

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed good based on available pictures and morphometric measurements.

The morphology of the parasite observed in the histopathological evaluation of the lungs as well as associated lesions are typical of an infection with a pseudaliid nematode of the genus *Halocercus* (*H. monoceris* has been reported in monodontidae). The nematodes observed in the larger airways of this

individual have a morphology that correspond to the pseudaliid nematode *Stenurus* sp. Pathogeny associated with these lungworms is typically associated to airway occlusion due to the physical presence of parasite within airways (Lair 2016, Measures 2018). The lung lesions observed in this individual were mild and did not affect its health considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition of the animal.

Trematodes have not been reported in beluga lymph node. In Hector's dolphins (*Cephalorhynchus hectori*), an adult trematode had a morphology corresponding to *Campula* spp and was noted to be encapsulated in the mesenteric lymph nodes, along with similarly encapsulated aggregates of trematode eggs associated with a strong macrophagic and fibrosing reaction (Hutton 1987). In general, digenean trematode of the genus *Campula* are associated with the liver, bile and pancreatic apparatus in odontocetes. They may cause variable level of lesions and their life cycle is poorly known. In this case, the encapsulated eggs in the lymph nodes were unlikely to have a negative health effect (Measures 2018).

The location and morphology of the encapsulated renal nematode is typical of the genus *Crassicauda* sp, a spirurid nematode found in mysticetes and odontocetes. *Crassicauda giliakiana* is generally site specific and associated to the urogenital system (Measures 2018). Although significant lesions can sometimes be associated with this parasite, in this animal the small proportion of renal tissue affected did not negatively impact its health.

Thyroid follicular cysts have been described in both arctic (Hudson Bay) and St. Lawrence beluga populations. They did not appear to be correlated with age and did not appear to have a clinical implication nor a clear suspected etiology (Mikaelian 2003).

Pituitary cysts have been described in cetacean species (Cowan 2008) and this observation is not considered to be significant.

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes. Likewise, adrenocortical nodular hyperplasia has been reported in adult (>15 years old) beluga whales from the St. Lawrence estuary and is stipulated to be part of a normal aging process or to alternatively constitute a regenerative response to degenerative lesions (Lair, 1997).

The scarce presence of spermatozoids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989).

Pharurus pallasii is the nematode commonly found in the middle-ear and sinuses of beluga. As they are usually not associated with lesions, they are considered an incidental finding (Lair 2014). Although precise morphology of the gastric nematodes is lacking, nematodes from the family Anisakidae are found in cetacean stomach. *Anisakis simplex* is the most prevalent gastric helminth in St. Lawrence beluga whales and is usually found in the first compartment of the stomach, while *Contracaecum* sp. were the most prevalent and abundant in beluga whales sampled from the Mackenzie River Delta (Lair 2016, Measures 1995, Wazura 1986).

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

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DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Émilie L. Couture, Dr. Rozenn Le Net, Hendrickson Island on the 2016-07-08.

Sex: Male

Age: ND

Weight: ND

Epaxial muscle weight (L1-L12): 29 kg

Body score (subjective): good

Length: 432 cm

Carcass condition score: 2

External examination

An adult male, white in coloration is examined. Multifocal, slightly depressed circular lesions with an irregular surface are noted mainly on the dorsal face. Approximately 50 of those lesions were seen and their size ranged between 1 and 2 cm in diameter.

Internal examination

Brain case: Over 100 nematodes were found in the brain case. They are 2 to 3 cm in length and less and 1 mm in diameter. It must be noted that the right earbone was cracked open, close to the location where the nematodes were found.

Lungs: One fragment of a black and white nematode measuring approximately 0.5 mm in diameter is noted in a small airway of the left lung.

Spleen: An accessory spleen of 2 cm x 4 cm is adjacent to the spleen.

Mesenteric lymph nodes: There are 4 nodules located in several mesenteric lymph nodes. They are white and firm. They contain a necrotic material in the center.

Right kidney: In the middle of the parenchyma, there is a cyst of 3 cm in diameter. It is delimited by a white capsule of approximately 1 mm in thickness. It contains a clear fluid as well as the remains of a nematode of 10 to 15 cm in length.

Stomach and intestines: No food material is noted in the digestive tract. Several nematodes are found in the first compartment of the stomach. Their diameter ranges from 2 to 4 mm and their length range from 7 to 12 cm. The intestines are moderately distended with bile coloured fluids.

The following structures were without noticeable lesions: Shoulder joints, muscles, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, thyroid gland, adrenal glands, heart, liver, pancreas, lymph nodes (tracheobronchic, mediastinal), urinary system (left kidney and bladder), reproductive system (testes and epididymis), esophagus.

HISTOPATHOLOGY

Skin and blubber (A): The demarcation between the stratum externum and the stratum intermedium is irregular.

Thyroid (C): A single large follicle, 1 cm in diameter is noted on the observed sections. It is lined by a cuboidal epithelium and surrounded by a fine strand of conjunctive tissue.

Lungs (D to I): Multifocally, infiltrations of eosinophils are seen within arteriolar walls (e.g. D-m, p, E-p). Mild to moderate interstitial infiltration of macrophages and eosinophils are noted surrounding blood vessels, bronchioles and alveoli. These infiltrations are sometimes associated with intraluminal accumulation of eosinophils and alveolar macrophages, and more rarely, small hemorrhages. Cut sections of a 0.1 mm min diameter nematode are seen within an empty alveole near a punctual foci of interstitial macrophagic inflammation (H-m). Focal foci of fibroplasia and mixed macrophagic-eosinophilic inflammation are also noted (H-p). In a locally extensive affected region (F-m), there is a focal accumulation of neutrophils within a bronchiole.

Lymph node (J to L): A 7 mm in diameter nodule is present on a section, with a thick fibrous capsule. Beneath the capsule, a ring of cellular debris and mineralized material partially washed away by slide preparation surround a center of densely packed, disorganized fibrous material. Clefts of granulous, sometimes mineral material as well as scarce trematode eggs are seen within this center (J). There is mild fibrosis on certain sections, including in the center of cortical lymphoid clusters, as well as rare accumulation of eosinophils within the lymphoid tissue, sometimes associated with granular eosinophilic material (K, L).

Reproductive tract (L, M): No sperm is stored in the epididymis (L), and there is not significant spermatogenesis within the testis (M)

Kidneys (N): A few collecting tubules are distended with proteinaceous fluid. A thick fibrous capsule adhered to the superficial portion of a section, that contains section of a 2 mm thick nematode with a thick, serrated pigmented cuticle.

Pituitary gland (P): Multiple (5-6) cystic structures or grouping of large follicles of a maximum of 1 mm in diameter and lined by are noted on the examined section, lined with a cuboidal to flat epithelium. These structures are filled with an eosinophilic liquid.

Adrenal (S to Y): On U (right adrenal) and V (left adrenal, 1.5 mm in diameter), there is an encapsulated nodular congregation of cells with a vast acidophilic cytoplasm sometimes containing acidophilic granules. There is a mild anisokaryosis. These structures do not seem to compress the surrounding parenchyma, nor do they distort the cortical thickness. Small scattered cystic structures of less than 1 mm in diameter are noted, and usually consist of a cluster of large follicles containing an eosinophilic liquid and separated by fine strands of conjunctive tissue (S, T).

No significant lesions on the examined sections from: muscle (A), pancreas (A), heart (B), central nervous system (P, Q, R), spleen (M), bladder (O), duodenum (O). The liver was not available for evaluation.

Histomorphologic diagnostics:

- Focal thyroid follicular cyst
- Mild multifocal chronic-active eosinophilic bronchopneumonia with nematodiasis
- Nodular lymph node trematode egg encapsulation
- Inactive spermatogenesis
- Focal encapsulated renal nematodiasis
- Multifocal small adenohipophysis cyst
- Bilateral adrenal cortical nodular hyperplasia
- Small, rare adrenocortical cysts

Resident:

Émilie L. Couture, DMV, IPSAV, DES

Pathologist:

Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2016-09
PATHOLOGY NO.: P3475-16

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested off of on 2016-07-14. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Halocercus* sp.)**
2. **Renal nematodiasis (*Crassicauda giliakiana*)**
3. **Gastric nematodiasis (Anisakidae)**
4. **Inactive spermatogenesis**
5. **Adrenocortical cyst**
6. **Thyroid fibrosis**
7. **Renal medullar mineralization**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed excellent based on available pictures and morphometric measurements.

The morphology of the parasite observed in the airways as well as associated lesions are typical of an infection with a pseudaliid nematode of the genus *Halocercus* (*H. monoceris* has been reported in monodontidae). In this case, the lesions were extremely discrete and localised, and granuloma formation and nematode mineralization are indicative of the chronic presence of this parasite. Considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of

the lung and the good general condition of the animal, this finding likely did not impact the general health of this animal (Lair 2016, Measures 2018).

The location and morphology of the encapsulated renal nematode is typical of the genus *Crassicauda* sp, a spirurid nematode found in mysticetes and odontocetes. *Crassicauda giliakiana* is generally site specific and associated to the urogenital system (Measures 2018). Although significant lesions can sometimes be associated with this parasite, in this animal the small proportion of renal tissue affected did not negatively impact its health.

Although precise morphology of the gastric nematodes is lacking, nematodes from the family Anisakidae are found in cetacean stomach. *Anisakis simplex* is the most prevalent gastric helminth in St. Lawrence beluga whales and is usually found in the first compartment of the stomach, while *Contracaecum* sp. were the most prevalent and abundant in beluga whales sampled from the Mackenzie River Delta (Lair 2016, Measures 1995, Wazura 1986).

The scarce presence of spermatozooids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989)

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes (Lair, 1997).

Thyroid interstitial fibrosis has been reported in cetaceans, notably in bottlenose dolphins, and the clinical significance of this finding was unknown (Cowan, 2006). Mild renal mineralization is also considered incidental.

Please note that lesions deemed associated with the harvesting method are not described in this report.

References

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- Lair S, Beland P, De Guise S, Martineau D. Adrenal hyperplastic and degenerative changes in beluga whales. *Journal of Wildlife Diseases*. 1997 Jul;33(3):430-7.
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DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Émilie L. Couture, Dr. Rozenn Le Net, on Hendrickson Island on the 2016-07-14.

Sex: Male

Age: ND

Weight: Not recorded

Epaxial muscle weight (L1-L12): 28 kg

Body score (subjective): good

Length: 427 cm

Carcass condition score: 2

External examination

An adult male, white in coloration is examined. Multifocal, slightly depressed circular lesions with an irregular surface are noted mainly on the dorsal face. Approximately 50 of those lesions were seen and their size ranged between 1 and 2 cm in diameter.

Internal examination

Kidneys: A nematode is found in a large collecting duct of one of the kidneys. It is spiral-shaped, of a mildly translucent dark yellow coloration and of approximately 25 cm in length and 2 mm in diameter.

Urinary bladder: At the apex of the bladder, surrounding urachus scar, there are around 20 dark red to black punctuations of 1 mm in diameter. They are limited to the mucosa.

Stomach and intestines: No food material is noted in the digestive tract. One nematode is found in the first compartment of the stomach. It is about 2 mm in diameter and 8 cm in length. The third compartment of the stomach and the intestines are slightly distended with bile coloured fluids.

The following structures were without noticeable lesions: shoulder joints, muscles, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, thyroid gland, adrenal glands, lungs, heart, liver, pancreas, spleen, lymph nodes (tracheobronchic, mediastinal, mesenteric), reproductive system (testes and epididymis), esophagus.

HISTOPATHOLOGY

Thyroid (B): There is noticeable differences in follicle size through the examined slides. Follicles also have irregular, concave borders, giving a 'collapsed' appearance. There is diffuse interstitial fibrosis between follicles.

Muscle (C): The sarcoplasm of four muscular cells is partially or totally replaced by fine granular material. On PAS coloration, this granular material stains dark magenta (glycogen reserves)

Lungs (D to L): Mild, multifocal and discrete interstitial infiltration of mononuclear cells (mainly macrophages), sometimes associated with alveolar presence of proteinaceous liquid, alveolar macrophages and eosinophils. Cut sections of nematodes, 0.1 mm in diameter, are observed (D-m, G-

p, H-s, I-s), in association with macrophagic interstitial accumulation as well as eosinophils and rarely neutrophils within airways (bronchioles, alveoles). The nematode viscera appear degenerate, and even mineralized in some sections (I-s). In the latter, it is surrounded by concentric accumulations of inflammatory cells and fine strands of conjunctive tissue.

Reproductive tract (K, N): No sperm is stored in the epididymis (N), and there is not significant spermatogenesis within the testis (K).

Pancreas (L): In some regions, the acinar cell nucleus appears condensed and the cytoplasm mildly distended with vacuoles.

Kidneys (M): Discrete, multifocal mineralization foci are noted in the renal medullar interstitium.

Bladder (N): The bladder epithelium is eroded multifocally. There is no associated inflammatory reaction (artefactual).

Adrenal glands (S to X): Cortical cysts of 0.5 to 2 mm in diameter are noted lined by a simple squamous epithelium (T, W). A bigger cyst of 5 mm in diameter and surrounded by a thick fibrous capsule is also noted in the cortex (U). Multiple mineralization foci are noted on the interior margins of the capsule, as well as clusters of extracellular yellow pigment deposition and occasional clear clefts that infiltrate the capsule's connective tissue. Pigments are moderately positive on PAS coloration. The surrounding parenchyma does not appear to be compressed

No significant lesions on the examined sections from: heart (A), skin and blubber (A), Lymph nodes (J, K), liver (L), spleen (L), duodenum (O), central nervous system (P, Q, R), pituitary gland (P).

Histomorphologic diagnostics

8. Diffuse, mild thyroid fibrosis
9. Possible degraded muscle sarcocystosis
10. Chronic-active, mild, multifocal discrete verminous bronchopneumonia (*Halocercus* sp.)
11. Inactive spermatogenesis
12. Mild interstitial renal medullar mineralization
13. Adrenocortical cyst and lipofuscin deposition
14. Pituitary cholesterol clefts?

Resident:

Émilie L. Couture, DMV, IPSAV, DES

Pathologist:

Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2016-10
PATHOLOGY NO.: P3476-16

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested off of on 2016-07-14. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Halocercus* sp., *Stenurus* sp.)**
2. **Thyroid cyst**
3. **Lymph node fibrosis**
4. **Inactive spermatogenesis**
5. **Granulomatous gastritis, unknown etiology**
6. **Renal medullar mineralization**
7. **Pituitary psammoma bodies**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed good based on available pictures and morphometric measurements.

The nematodes observed in the small airways of the lungs during gross necropsy are likely pseudaliid nematodes of the genus *Stenurus* sp. (*S. arctomarinus* has been reported in monodontidae). Pathogeny associated with these lungworms is typically associated to airway occlusion due to the physical presence of parasite. No gross or microscopic evidence of pathology was evident in this case. The morphology of the parasite observed histologically in the airways as well as associated lesions are typical of an infection

with a pseudaliid nematode of the genus *Halocercus* (*H. monoceris* has been reported in monodontidae). In this case, the lesions were generally discrete and localised except in lung sections where nematodes were noted. Nematode mineralization is indicative of the chronic presence of this parasite. Considering the absence of gross lesions, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition, this finding likely did not impact the general health of this animal (Lair 2016, Measures 2018).

Thyroid follicular cysts have been described in both arctic (Hudson Bay) and St. Lawrence beluga populations. They did not appear to be correlated with age and did not appear to have a clinical implication nor a clear suspected etiology (Mikaelian 2003).

An increased deposition of connective tissue in human lymph nodes is reported as one of various possible degenerative changes associated with senescence and appears mostly noted in superficial inguinal nodes (Ahmadi 2013).

The scarce presence of spermatozoids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989)

Psammoma bodies have been described in cetacean species (Cowan 2008) and this observation is not considered to be significant, such as the renal mineralization.

The association of the gastric lesions with macroscopic observations is uncertain. This finding likely did not affect the general health of this animal that had a good body condition.

Please note that lesions deemed associated with the harvesting method are not described in this report.

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- Stewart BE, Stewart RE. *Delphinapterus leucas*. Mammalian Species. 1989; (336): 1-8.

DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Émilie L. Couture, Dr. Rozenn Le Net, Hendrickson Island 2016-07-08.

Sex: Male

Age: ND

Weight: Not recorded

Epaxial muscle weight (L1-L12): 25 kg

Body score (subjective): good

Length: 399 cm

Carcass condition score: 2

External examination

An adult male, white in coloration is examined. Multifocal, slightly depressed circular lesions with an irregular surface are noted mainly on the dorsal face. Approximately 50 of those lesions were seen and their size ranged between 1 and 2 cm in diameter.

Internal examination

Thyroid gland: Two cystic structures are found on the surface of the left lobe of the thyroid. They are delimited by a white capsule and measure approximately 1.5 cm in diameter. When cut open, both cysts contain a thick, cloudy and white liquid material.

Lungs: Occasional black and white nematodes measuring approximately 0.5 mm in diameter and a few centimeters in length are noted in the small airways of both lungs.

Stomach and intestines: No food material is noted in the digestive tract. Multifocally, the mucosa of the second stomach compartment is dark brown. This coloration is superficial, irregular in shape and does not appear to be associated with other lesions. The third compartment of the stomach and the intestines are slightly distended with bile coloured fluids.

The following structures were without noticeable lesions: shoulder joints, muscles, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, lungs, heart, liver, pancreas, spleen, lymph nodes (tracheobronchic, mediastinal, mesenteric), urinary system (kidneys and urinary bladder), reproductive system (testes and epididymis), esophagus.

HISTOPATHOLOGY

Thyroid (C): A portion of a large cyst with a thick fibrous capsule is seen. Strands of fibrous tissue stemming from the capsule extend in the adjacent parenchyma. The cystic structure is lined with a single, flat to cylindrical with ciliated border epithelium. Mineralized particles are seen within the amorphous to finely granular acidophilic content of the cyst.

Lungs (D to I): Depending on the sections, the lesions range from mild and discrete to marked and locally expansive. Interstitial infiltration of inflammatory cells (Mononuclear predominantly, sometimes mixed with eosinophils), sometimes accompanied by intraluminal accumulation of macrophages, eosinophils and rarely, large number of erythrocytes are noted (alveoli, bronchioles). Multiple sections of nematodes, including female nematodes of 0.15 mm in diameter and containing larvae and others 0.1 mm in diameter, are seen within bronchioles and alveoli (D-p). Focal areas of fibroplasia are surrounded by mononuclear cell infiltration (E-m). Degenerate and mineralized sections of nematodes are also noted in association with mononuclear infiltration (E-p).

Lymph nodes (J, K): On one section (J), extensive regions of fibrosis are noted throughout the lymphonodular parenchyma, leaving scarce lymphoid aggregates visible. Focally, eosinophil infiltrate a lymph node capsule (K).

Reproductive tract (L): No sperm is stored in the epididymis, and there is minimal spermatogenesis within the testis.

Kidneys (N): On one section, 2 small foci of mineralization are seen within the medullar interstitium.

Stomach (P): There is approximately four foci of lamellar aggregation of inflammatory cells (mononuclear, eosinophils) layered with fine strands of conjunctive tissue. In the center of these aggregations is not always discernible, but in the aggregation located within the glandular tissue, appears to be constituted of a disorganised clump of mildly basophilic spindle cells. The other aggregations are located in the submucosa.

Pituitary (Q): Rare punctual foci of mineralisation are noted.

No significant lesions on the examined sections from: heart (A), skin and blubber (A), muscle (B), thymus (K), pancreas (M), spleen (M), liver (M), bladder (O), duodenum (O), central nervous system (Q,R, S), adrenals (T to Y)

Histomorphologic diagnostics

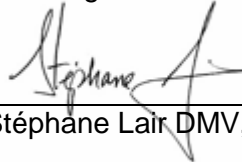
- Thyroid cyst
- Chronic-active, mild to moderate, multifocal verminous bronchopneumonia (*Halocercus* sp.)
- Lymph node fibrosis
- Inactive spermatogenesis
- Focal, mild interstitial renal medullar mineralization
- Focal granulomatous gastritis of unknown cause
- Pituitary psammoma bodies

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM



Necropsy report

From

Canadian Wildlife Health Center
 Faculté de médecine vétérinaire, Université de Montréal
 3200, rue Sicotte
 Saint-Hyacinthe, Québec J2S 2M2
 Tel: (450) 773-8521 ext. 8346



ARHI-DL-2016-11
 PATHOLOGY NO.: P3477-16

HISTORY AND GENERAL INFORMATION

Submitted to (send report to):

Lisa Loseto
 Central and Arctic Region
 Freshwater Institute
 Fisheries and Oceans Canada / Canada
 501 University Crescent, Winnipeg
 MB R3T 2N6, Canada.
 Tel: 208 983 5135

An adult male beluga whale was harvested off of on 2016-07-14. The whale was shot in the head and part of the dorsal brain case was severely damaged. A complete necropsy was performed once the animal was towed to Hendrickson Island shortly after.

DIAGNOSTICS AND INTERPRETATION

Final diagnostics:

1. **Verminous bronchopneumonia (*Halocercus* sp., *Stenurus* sp.)**
2. **Thyroid gland – adenomatous hyperplasia**
3. **Thyroid cysts (< 1 mm)**
4. **Lymph node fibrosis**
5. **Inactive spermatogenesis**
6. **Unilateral adrenal cortical adenoma**
7. **Adrenocortical cysts**

Interpretation and comments:

None of the conditions noted in this individual negatively affected its general condition. This animal's body condition was deemed good based on available pictures and morphometric measurements.

The nematodes observed in the gross examination of the lungs are likely pseudaliid nematodes of the genus *Stenurus* sp. (*S. arctomarinus* has been reported in monodontidae). The morphology of the parasite observed on histologic sections as well as associated lesions are typical of an infection with a pseudaliid nematode of the genus *Halocercus* (*H. monoceris* has been reported in monodontidae). In this case, while some lesions were discrete and localised, they tended to be more locally extensive especially

in sections where nematodes were noted. Granulomatous reactions and nematode mineralization are indicative of the chronic parasitic presence. Considering the mild gross lesions observed, the small proportion of affected parenchyma on microscopic examination of the lung and the good general condition, this finding likely did not impact the general health of this animal (Lair 2016, Measures 2018).

Thyroid follicular cysts and adenomatous hyperplasia have been described in both arctic (Hudson Bay) and St. Lawrence beluga populations. Cysts did not appear to be correlated with age and did not appear to have a clinical implication nor a clear suspected etiology, while the volume of adenomatous hyperplasia was positively correlated with age (Mikaelian 2003).

An increased deposition of connective tissue in human lymph nodes is reported as one of various possible degenerative changes associated with senescence and appears mostly noted in superficial inguinal nodes (Ahmadi 2013).

The scarce presence of spermatozoids at this time of the year corresponds to the time of harvest occurring after the end of the breeding season (O'Brien 2008, Stewart 1989)

Adrenocortical cysts are often noted in mature beluga whales. Their size is positively correlated with age and they have been described both in the St. Lawrence estuary and Hudson Bay populations. They have been postulated to be part of a normal aging process. However, adrenocorticolytic xenobiotics as well as stress have also been suggested as possible underlying causes. Likewise, adrenocortical nodular hyperplasia has been reported in adult (>15 years old) beluga whales from the St. Lawrence estuary and is stipulated to be part of a normal aging process or to alternatively constitute a regenerative response to degenerative lesions (Lair, 1997).

The presence of macrophages laden with lipochrome in the perivascular spaces and within neurons are reported changes associated with senile atrophy in domestic animals. Observed changes appeared localised and mild and were probably not significant clinically (Cantile 2016).

Please note that lesions deemed associated with the harvesting method are not described in this report.

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DETAILED REPORT

GROSS EXAMINATION

Necropsy done by Dr. Émilie L. Couture, Dre Dr. Rozenn Le Net, Hendrickson Island on the 2016-07-08.

Sex: Male

Age: ND

Weight: ND

Epaxial muscle weight (L1-L12): 31 kg

Body score (subjective): excellent

Length: 412 cm

Carcass condition score: 2

External examination

An adult male, white in coloration is examined. Multifocal, slightly depressed circular lesions with an irregular surface are noted mainly on the dorsal face. Approximately 50 of those lesions were seen and their size ranged between 1 and 2 cm in diameter.

Internal examination

Thyroid gland: The thyroid is asymmetric with the right lobe being approximately two thirds of the left lobe in size. There are approximately 20 nodules in the parenchyma. They are light yellow, firm, regular in shape but not always well delimited. They are distributed multifocally in both lobes, mainly at the surface of the dorsal aspect.

Lungs: In both lungs, there are a few dozens indurations of a few millimetres in diameter (from 2 to 6 mm approximately). They are multifocally and randomly distributed. They are firm, irregular in shape, light yellow in color not well delimited. Occasional black and white nematodes measuring approximately 0.5 mm in diameter and a few centimeters in length are noted in the small airways of both lungs.

Stomach and intestines: No food material is noted in the digestive tract. The third compartment of the stomach and the intestines are slightly distended with bile coloured fluids.

The following structures were without noticeable lesions: shoulder joints, muscles, central nervous system (cerebrum, cerebellum and cerebral trunk), pineal gland, adrenal glands, lungs, heart, liver, pancreas, spleen, lymph nodes (tracheobronchic, mediastinal, mesenteric), urinary system (kidneys and urinary bladder), reproductive system (testes and epididymis), esophagus.

HISTOPATHOLOGY

Thyroid (C): Multiple nodules are present on both sections observed. They range from 0.5 mm in diameter to 3-4 mm. They are encapsulated, compress the surrounding parenchyma and contain dense aggregations of follicular cells where rare to multifocal small follicles may still be found. A few nodules are composed of a looser arrangement of irregularly shaped follicles of varying sizes. A group of non-

encapsulated enlarged follicles (0.5-1 mm in diameter), lined by a squamous epithelium and containing a dense colloid is also present

Lungs (D to I): Depending on the sections, the lesions range from mild and discrete to marked and locally expansive. Interstitial infiltration of inflammatory cells (Mononuclear predominantly, sometimes mixed with eosinophils), sometimes accompanied by intraluminal accumulation of macrophages, eosinophils and rarely, large number of erythrocytes are noted. Within the most affected areas, multiple sections of nematodes, 0.1-0.15 mm in diameter, including females bearing larvae, are seen within bronchioles and alveoli (D-m, D-p*, E-s, G-p, H-s, I-M, I-p). Focal accumulations of neutrophils and giant multinucleated cells may be noted in the nematodes surrounding. Degenerate and mineralized sections of nematodes are also noted in association with inflammatory cell infiltration, including lamellar organisation of macrophages with thin layers of conjunctive tissue (D-p). A mineralization foci is seen within an arteriole (E-s). Focal areas of fibroplasia are surrounded by mononuclear cell infiltration (F-m)

Lymph nodes (J): A focal eosinophil aggregation is noted within a lymphoid follicle. All lymph node sections generally appear to have an increased amount of fibrous tissue.

Liver (K): The periportal hepatocytes frequently have cytoplasmic accumulation of dark pigments.

Reproductive tract (L): No sperm is stored in the epididymis, and there is minimal spermatogenesis within the testis. Multifocal foci of mineralisation are seen within the epididymal epithelium.

Central nervous system (O, P, Q): Mild perivascular and meningeal hemorrhages are noted on all slides (gunshot near braincase). Mild, focal perivascular mononuclear cell accumulation (P)

Adrenals (R to Y): The medulla appears generally enlarged on all sections when compared with other individuals. The extremity of the cranial right adrenal gland is delimited by a thick fibrous strand. Within this fibrous strand, there is a nodular accumulation of clear clefts (cholesterol clefts) (R). Multiple cystic structures, from 1 mm to 4 mm are noted through the parenchyma, they are often composed of smaller cysts separated by fine strands of conjunctive tissue (spider web) (R, S, W, X). There is an encapsulated nodular congregation (5 mm in diameter) cells with a vast acidophilic cytoplasm, sometimes containing acidophilic granules (W). There is a moderate anisocytosis and anisokaryosis. These structures do not seem to compress the surrounding parenchyma, nor do they distort the cortical thickness.

No significant lesions on the examined sections from: heart (A), muscle (B), skin and blubber (A), pancreas (K), spleen (K), kidneys (M), bladder (N), duodenum (N), hypophysis (O)

Histomorphologic diagnostics

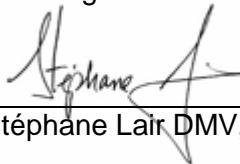
- Multifocal adenomatous hyperplasia of the thyroid gland
- Thyroid cysts
- Chronic-active, mild to moderate, multifocal verminous bronchopneumonia (*Halocercus* sp.)
- Lymph node fibrosis and focal eosinophil aggregation
- Inactive spermatogenesis
- Focal cerebral perivascular lipofuscinosis
- Unilateral adrenal cortical adenoma
- Adrenocortical cysts

Resident:



Émilie L. Couture, DMV, IPSAV, DES

Pathologist:



Stéphane Lair DMV, Diplomate ACZM

APPENDIX VII – FOOD-BORNE PARASITE TESTING - 2015 AND 2016

Beluga whale health assessment and Food-borne parasite testing from 2015 to 2016.

Samples were collected from the Eastern Beaufort Sea beluga population, in collaboration with the annual harvest in Hendrickson Island and East Whitefish, Northwest Territories, Canada. A total of 34 whales were sampled in 2015 and 2016. Blood samples were collected from neck or tail vessel transections, centrifuged at 3000 rpm for 15 min, serum was recovered and stored at -80°C until further analyses. Approximately 100 g of heart, brain, tongue and diaphragm tissues were collected and stored at -20°C until further analyses.

Recent reports of the microscopic parasite *Toxoplasma gondii* in Arctic marine mammals, including beluga in the ISR, have raised concerns that this parasite may affect the health of beluga, or people who harvest beluga. This parasite has been reported in a wide range of wildlife in the Canadian Arctic. Disease associated with this parasite is called toxoplasmosis. While usually asymptomatic, it can cause brain inflammation (encephalitis) in many marine mammals, including beluga in the St. Lawrence River.

Serum samples were tested for antibodies to *Toxoplasma gondii* using the commercial Enzyme linked immunosorbent assay (ELISA) kit (Id Screen Toxoplasmosis Indirect Multi-species, IdVet Innovative Diagnostics. Grabels, France) as gold standard, an in-house modified agglutination test (MAT; Al-Adhami *et al*, 2016) and immunofluorescence antibody test (IFAT). From 33 serum samples, only one (AREW15-06) tested doubtful by ELISA and positive by IFAT. The samples left tested negative for *T. gondii*.

Brain and heart tissue were tested by the Magnetic capture (MC) DNA extraction and qPCR (Opsteegh *et al*, 2010) for detection of *T. gondii* DNA. This technique is the new European Food Safety Authority gold standard for detecting *Toxoplasma* in meat and allows us to analyze 1000 times more meat per animal than previous methods. From 31 heart samples and 23 brain samples, only one heart sample (ARH15-06) tested positive by this technique.

Table 6. Beluga whale samples tested by serological and molecular techniques in 2 different years of harvesting. Results are expressed as number of positives/total of samples.

Year	ELISA	MAT	IFAT	MCqPCR heart	MCqPCR brain
2015	1/18	0/18	1/18	1/15	0/13
2016	0/15	0/15	0/15	0/16	0/10

Detection of *Trichinella* larvae in muscle. *Trichinella* is a roundworm food-borne parasite. The test was performed by a double separatory funnel digestion. Diaphragm and tongue samples were artificially digested in a pepsin/HCl solution at 37°C in a magnetic stirrer for 1.5 hours. Digestion fluid was poured through a sieve into a separatory funnel and tap water at RT was added to stop the digestion. The sediment was drained into a Petri dish and examined on a stereomicroscope for the presence of *Trichinella* larvae. The last step was repeated twice. We did not detect *Trichinella* in tongues and diaphragm muscles examined from belugas harvested in 2015-2016.