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**CANINE DISTEMPER VIRUS IN THE SEA OTTER (*ENHYDRA LUTRIS*) POPULATION IN WASHINGTON STATE, USA**

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**ABSTRACT:** **Before 2001, all serosurveys for morbilliviruses in sea otters (*Enhydra lutris*) in California, Washington, and Alaska, US, documented a 0% seroprevalence**. **The first published serologic detections of morbillivirus in sea otters occurred in 2001–02 in live-captured Washington sea otters, with a documented 80% seroprevalence**. We conducted a retrospective study of sea otter cases from 1989 to 2010 compiled at the US Geological Survey, National Wildlife Health Center to identify cases of morbilliviral disease in Washington sea otters and to characterize the disease using immunohistochemistry, reverse transcription (RT)-PCR, genetic sequencing, virus isolation, and serology. We identified six cases of morbilliviral disease and 12 cases of morbilliviral infection in this population of sea otters during 2000–10. **Significant histologic findings included inflammation in the white and gray matter of the brain characterized by lymphoplasmacytic perivascular cuffing, neuronal necrosis, and satellitosis in gray matter and by spongiosis, myelin degeneration, spheroids, and gemistocytes in white matter. Intranuclear and intracytoplasmic viral inclusion bodies were found in neurons, Purkinje cells, and glia.** Immunohistochemistry for canine distemper virus (CDV) showed positive staining in neurons, glial cells, and cell processes. A pan-morbillivirus RT-PCR with subsequent restriction endonuclease digestion or sequencing identified CDV. Virus isolation was not successful. Two sea otters with morbilliviral encephalitis showed greater antibody titers to CDV than phocine distemper virus. **Histologic changes were confined to the central nervous system and resembled neurologic canine distemper in domestic dogs.** Cases of sea otters with morbilliviral infection without histologic changes could represent early infections or incompletely cleared sublethal infections. **We found that morbillivirus was present in the Washington sea otter population as early as 2000, and we provide a description of the pathology of canine distemper in sea otters.**

**Study Design**:

* Retrospective analysis of beachcast Washington sea otters submitted for necropsy to the USGS National Wildlife Health Center between 1989-2010 (n=71).
* Cases were reviewed for histopathologic findings compatible with morbilliviral disease, specifically for nonsuppurative encephalitis of morbilliviral or unknown origin, interstitial pneumonia, or an unexplained cause of death
* An additional criterion for selection was otter deaths associated in time or place with an otter with morbilliviral disease

**Goal:**

* Identify the virus associated with morbilliviral disease in Washington sea otters and document morbillivirus-associated pathology

**Key Points:**

* Morbilliviruses gained prominence as a cause of mass mortality in marine mammals when phocine distemper virus (PDV) decimated European harbor seals in the North Atlantic Ocean in 1988
  + Epidemics due to canine distemper virus (CDV) of terrestrial origin had occurred previously in land-locked Baikal seals in 1987 and subsequently in Caspian seals in 2000, but PDV, although related, was found to be antigenically and genetically distinct from CDV
  + Other morbilliviruses known to cause disease and mortality in cetaceans, including dolphin morbillivirus and porpoise morbillivirus, are considered cetacean morbilliviruses (CMVs)
* Morbilliviral disease, whether PDV, CMV, CDV, or human measles virus, results in acute epithelial and lymphoid system damage, potentially followed by subacute-to-chronic neurologic disease and opportunistic infections
* In this study, 19 of 71 sea otter necropsy cases met the selection criteria for further investigation for morbilliviruses
  + 6 of the 19 sea otters had histopathologic findings consistent with morbilliviral disease, all limited to the brain
* This study describes the pathology of morbillivirus in sea otters and identifies CDV as the causative virus by RT-PCR, sequencing, and IHC
  + Serology was also supportive
  + Virus isolation was negative for all submitted tissues
  + Pathology was confined to the central nervous system and resembled neurologic CDV infection in other wild and domestic species
  + The principal diagnostic features were prominent inflammation in both the white and gray matter of the brain, particularly affecting the brainstem, cerebellar medulla, and corpus callosum
* The inflammation was characterized by astrocyte hypertrophy and spongy change in white matter and the presence of intracytoplasmic and intranuclear inclusion bodies
  + The virus could be detected by RT-PCR in brain, and viral antigen could be visualized by IHC in inflammatory foci and sometimes was widespread in neurons, glia, and their processes
  + Virus was detected by RT-PCR in kidney from most sea otters with neurologic lesions and by IHC in renal tubular cells in one case
  + Syncytia were seen occasionally but only in the leptomeninges
  + Viral inclusion bodies usually were few, intracytoplasmic, and found in the brain
* Pathology associated with morbilliviral disease in sea otters differed from that observed in pinnipeds and cetaceans dying during morbilliviral epizootics
  + In those epizootics, severe systemic disease accompanies neurologic lesions so that viral inclusions and antigen are readily detected within the lung, in lymphoid tissue, and in other epithelial sites
  + Localized neurologic morbilliviral disease in the absence of systemic lesions has been reported in individual cetacean mortalities, most notably in Mediterranean striped dolphins (*Stenella coeruleoalba*) in years following the first epizootic in that species and region
    - In striped dolphin cases, lesions were confined to the central nervous system and resembled chronic localized morbillivirus encephalitis in dogs and terrestrial wildlife that survive the acute systemic stage of distemper
* Concurrent infections occur commonly in dogs and wildlife as a complication of morbillivirus infections
  + Opportunistic organisms gain footholds after lymphoid destruction in the systemic stage of the disease
* Morbillivirus was detected in tissues from sea otters with no lesions of morbilliviral disease
* Morbillivirus also was detected by RT-PCR in kidneys from 10 of 13 sea otters with no evidence of morbilliviral disease, and this was the only positive tissue in seven of these animals
* Morbillivirus was detected in 4 of 5 sea otters examined from the 2000 mortality event, but lesions indicative of morbillivirus were not found in any cases until 2004
  + In other words, morbillivirus was present in Washington sea otters dying during the 2000 event, but we have no definitive evidence that infection was associated with disease
* CDV seroprevalence declined to 3 of 30 (10%) in this population as of 2011, potentially leaving a large portion of the population again susceptible should a morbillivirus be reintroduced

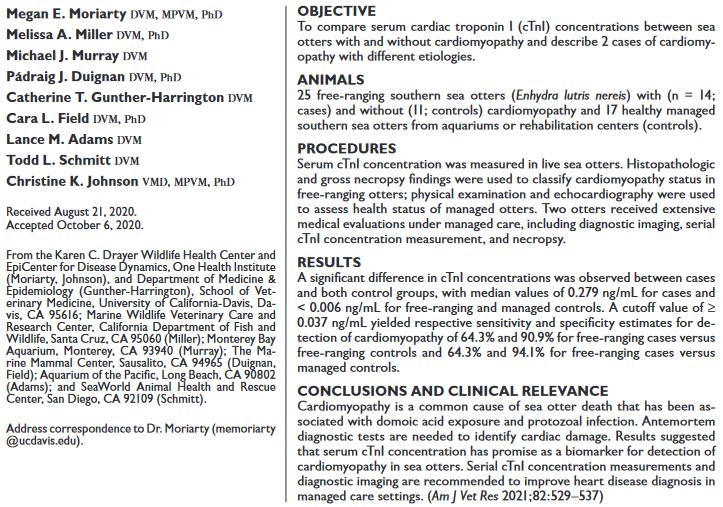
**TLDR:**

* Morbillivirus was present in the Washington sea otter population as early as 2000 but we have no definitive evidence that infection was associated with disease
* This study describes the pathology of morbillivirus in sea otters and identifies CDV as the causative virus by RT-PCR, sequencing, and IHC
  + Pathology was confined to the central nervous system and resembled neurologic CDV infection in other wild and domestic species

**Related Articles**

White CL, Lankau EW, Lynch D, Knowles S, Schuler KL, Dubey JP, Shearn-Bochsler VI, Isidoro-Ayza M, Thomas NJ. 2018. Mortality trends in northern sea otters (*Enhydra lutris kenyoni*) collected from the coasts of Washington and Oregon, USA (2002–15). *J Wildl Dis* 54:238–247.

Moriarty, M. E., Miller, M. A., Murray, M. J., Duignan, P. J., Gunther-Harrington, C. T., Field, C. L., ... & Johnson, C. K. (2021). Exploration of serum cardiac troponin I as a biomarker of cardiomyopathy in southern sea otters (Enhydra lutris nereis). *American Journal of Veterinary Research*, *82*(7), 529-537.



* Introduction:
  + Cardiomyopathy primary/contributing cause of death in 41% free-ranging sea otters that underwent necropsy from 1998-2012.
  + Multifactorial – Protozoal infection (*Toxoplasma gondii, Sarcocystis neurona*) and DA toxin exposure identified risk factors.
    - Domoic acid – Neurotoxin produced by harmful algal blooms, important cause of death in marine mammals along the Pacific Coast of North America.
      * Causes neurologic and GI disease in addition to cardiovascular effects.
      * Sea otters with DA associated CNS abnormalities often have cardiovascular lesions.
      * DA cardiomyopathy progressive, dilated cardiomyopathy end lesion.
      * No accurate antemortem clinical indicator has been identified for detection of acute DA toxicosis or chronic effects such as cardiomyopathy.
  + Cardiac troponins – Blood-based biomarkers of heart disease.
    - Cardiac troponin 1 – Part of a regulatory protein complex in the contractile fibers of cardiomyocytes that controls the calcium-mediated interaction between actin and myosin.
      * Highly conserved biomarker of myocardial injury, detectable by immunoassay with high sensitivity for myocardial damage.
      * Cardiac specificity, negligible presence in healthy individuals, rapid release, continued presence in circulation for days after damage.
    - Harbor seal pups that die during or after rehabilitation have been found to have a significantly higher antemortem serum cTn1 at admission vs those that survive after release.
* Objectives: Compare cTn1 concentration in antemortem serum samples from otters with cardiomyopathy and 2 groups without cardiac disease, describe cardiomyopathy of different etiologies in 2 sea otters.
* M+M:
  + Free-ranging otters stranded and died or were euthanized with cardiomyopathy were selected as cases.
    - All cases had microscopically confirmed moderate to severe cardiac disease.
    - Assessment included DA-associated brain abnormalities.
    - Cases also included a cardiac inflammation severity grade (mild, moderate, marked), predominant inflammatory cell type (lymphoplasmacytic or pleocellular), and whether protozoal cysts, schizonts, or zoites were present in heart, brain, or skeletal muscle.
  + Two other groups used as controls for comparison of serum cTn1 with cases.
    - Either died or were euthanized with no cardiac disease, DA toxicosis, or protozoal infection.
    - Or healthy managed sea otters from aquariums and rehab centers.
  + Blood samples collected, cTn1 concentrations measured by commercially available immunoassay.
* Results:
  + 14 wild otters with cardiomyopathy, 11 wild otters without cardiomyopathy, 17 healthy managed otters included.
    - All wild otters had cardiomyopathy and DA toxicosis as primary or contributing cause of death. Systemic protozoal disease primary or contributing cause of death in 4 cases.
    - cTn1 differed significantly between free-ranging cases and free-ranging controls, and between free-ranging cases and managed controls.
    - Used cutoff value of > 0.037 ng/mL.
    - For free-ranging cases vs free-ranging controls, resulted in 76% otters being correctly classified, 64.3% sensitivity and 90.9% specificity.
    - For free-ranging cases vs managed controls, resulted in 81% otters being correctly classified, 64.3% sensitivity and 94.1% specificity.
* Clinical case study 1: Cardiomyopathy associated with DA toxicosis:
  + 13yo F southern sea otter, stranded with end-lactation syndrome.
    - Grade 4/6 systolic murmur over mitral valve, regular rhythm.
    - Cardiac size (VHS) on radiographs measured normal, mild to moderate interstitial pulmonary pattern on radiographs.
    - Over next 7 months, thoracic radiographs revealed progressive cardiac enlargement, increasingly globoid cardiac silhouette, pulmonary venous distension.
    - ECG showed progressively reduced P wave amplitude, increased atrial and ventricular ectopy, occasional first and second degree AV block.
    - cTn1 initially low and peaked 9 months after stranding.
    - Appetite declined, progressed to congestive heart failure with ventricular bigeminal pattern.
    - Serum cTn1 decreased and the otter died a month later.
    - Necropsy – Severe, progressive DCM and biventricular heart failure.
      * Brain lesions consistent with severe subacute and chronic DA toxicosis.
* Clinical case study 2: Cardiomyopathy associated with a protozoal infection:
  + 10yo F southern sea otter, permanent resident at Monterey Bay Aq.
    - Chronic bilateral hip osteoarthritis. Otherwise apparently healthy.
  + Echo revealed mild to moderate thickening of mitral and tricuspid valves, serum cTn1 increased to 0.128 ng/mL.
  + Appetite decreased ~6 months later, cTn1 increased to 1.165 ng/mL.
  + Serum IgG vs S. neurona previously negative until this time, titer increased 64 fold.
  + 1 week later, neurologic signs, tremors, blepharospasm.
  + Serum cTn1 decreased to 0.123 ng/mL, IgG titer vs S. neurona increased.
    - Suggestive of acute infection. Within two days, progressed to seizures.
    - ECG revealed second degree AV block, thoracic radiographs showed cardiac enlargement. Otter died next morning.
  + Necropsy: Cardiac enlargement with marked focally extensive pallor of the left ventricular epicardium, extending to pulmonic trunk on right ventricle.
  + Nodular endocardiosis of both tricuspid and mitral valves.
  + Brain was edematous with cerebellar herniation.
  + Histology confirmed severe myelomeningoencephalitis and multifocal myocarditis associated *S. neurona* ID by IHC.
    - Muscles of respiration, pectoralis muscles, intercostalis muscles, diaphragm most severely affected.
* Discussion:
  + High sensitivity – Provides few false-negative results.
    - Fewer cardiomyopathy cases would be missed.
    - Good for disease screening, negative results rule out cardiac disease.
    - False-negative results of the cTn1 immunoassay could be common in sea otters with cardiac disease.
      * cTn1 has short half-life in serum.
      * Only patients with ongoing myocardial injury can have high serum cTn1.
  + High specificity – Yields few false-positive results.
    - Fewer sea otters would be misclassified as having cardiomyopathy.
    - Ideal test for disease confirmation, whereby positive results rule in cardiomyopathy.
* Takeaways:
  + DA exposure and protozoal infection risk factors for cardiomyopathy, end stage is DCM.
  + Cardiac troponin 1 is not specific for a particular mechanism of myocardial injury.
  + Importance of evaluating cardiac and systemic causes of disease and interpreting serum cTn1 concentrations in conjunction with PE and diagnostic imaging results.
  + Serial measurement of serum cTn1 appears to be an important tool for understanding progression of cardiac disease in sea otters.
  + In both clinical cases described here, peak serum cTn1 concentration was detected several days to months before death.
    - Fluctuating cTn1 over time highlights importance of serial testing.

**Baseline urinalysis results in 32 healthy Antillean manatees (Trichechus manatus manatus)**

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**Abstract**:

OBJECTIVE To describe results of analysis of free-catch urine samples collected from Antillean manatees (Trichechus manatus manatus) under human care in the Caribbean.

ANIMALS 32 Antillean manatees in 5 Caribbean oceanaria and rescue centers.

PROCEDURES Urine samples were obtained by opportunistic free catch during physical examination or through the use of operant conditioning procedures. Urinalyses consisted of macro- and microscopic evaluations, biochemical analyses with test strips, and refractometry. Results were compared for manatees grouped on the basis of age, sex, and habitat.

RESULTS Urine samples were typically clear, straw colored, and alkaline (mean pH, 8.0); had a urinoid odor and low specific gravity (mean, 1.010); and had results on qualitative test strips that were consistently negative for the presence of glucose, bilirubin, ketones, proteins, nitrites, RBCs, and WBCs. Microscopically, the mean ± SD number of RBCs and WBCs/hpf was 0.5 ± 0.3 RBCs/hpf and 1.1 ± 1.5 WBCs/hpf. The presence of some epithelial cells and crystals was typical. Spermatozoa were found in urine from 1 of 15 sexually mature males, and parasite larvae and eggs were found in urine from 2 manatees.

CONCLUSIONS AND CLINICAL RELEVANCE Results of the present study yielded the first compilation of baseline urinalysis values in healthy Antillean manatees under human care, which, when combined with physical examination and other diagnostic procedures, can help in monitoring the health of these animals. We encourage the use of free-catch urine collection methods, as used in the present study, for routine urinalyses of manatees under human care in zoos, aquaria, or rescue centers.

**Summary**:

* Objective - describe the macroscopic, microscopic, and biochemical characteristics of free-catch urine collected through noninvasive medical operant conditioning in Antillean manatees under human care in the Caribbean
* M+M:
  + Urine collected under behavioral restraint in 32 manatees
  + UA – macroscopic, biochem, microscopic analysis
* Results/conclusion
  + Straw-colored urine - most common and considered clinically normal
  + urinoid odor - most common
  + negative for glucose, bilirubin, urobilinogen, ketones, nitrite, protein, blood, and leukocytes
  + 0-8 WBCs/hpf and 0-3 RBCs/hpf
  + Alkaline urine – different than other marine mammals but consistent with other herbivores
  + urine SG - 1.010 ± 0.007 (range 1.002 - 1.030)
    - poor concentrating ability

epithelial cells commonly observed

* + higher numbers of squamous epithelial cells in urine from females (vs males)
  + nematode-type larvae and eggs in urine in several animals
  + See summary below

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Graphical user interface, application

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ESOPHAGEAL MEASUREMENT OF CORE BODY TEMPERATURE IN THE FLORIDA MANATEE (TRICHECHUS MANATUS LATIROSTRIS)

Journal of Wildlife Diseases, 56(1), 2020, pp. 27–33

ABSTRACT: Cold-stress syndrome (CSS) is one of the leading natural threats to free-ranging Florida manatees (Trichechus manatus latirostris). Cold water exposure below the species’ acceptable physiologic range is a frequent occurrence for manatees during cold weather months causing CSS induced systemic illness and significant annual mortality. Although CSS is a commonly presented condition at manatee rehabilitation facilities, the core body temperatures in CSS manatees are currently unknown due to the lack of clinically applicable and accurate temperature measurement methodologies**. Our objective was to establish a clinically applicable measurement methodology of core body temperature in manatees.** A novel, minimally invasive temperature technique to obtain esophageal temperature by placing a temperature sensor through an oro-gastric tube was compared to current oral and nasal methods in 20 clinically healthy manatees. **Results identified the esophageal measurement as the best performing and most precise temperature methodology.** The superior performance of esophageal temperature measurements differed significantly from both nasal and oral measurements, while nasal and oral measurements did not differ when compared with each other. **The esophageal measurements were consistent with manatee core body temperature, facilitating generation of a reference interval for core body temperature in healthy manatees (35.0– 35.8 C).** Four CSS medical cases were evaluated with the newly validated esophageal temperature method, facilitating diagnosis of hypothermia. The application of this temperature measurement technique to CSS manatees in field or rehabilitation settings will help in understanding CSS pathophysiology, improve medical assessments during rehabilitation, and contribute to conservation efforts.

Intro

* Cold stress syndrome (CSS) is one of the leading threats to wild Florida manatees
* No accurate method for assessing core body temp in manatees exist
* objective was to establish a clinically applicable measurement methodology of core body temperature in manatees

M&M

* n = 20 healthy manatees
* Measurement of esophageal temps via orogastric tube with a digisense thermometer. This was compared to oral and nasal measurements
* Temperatures from 4 manatees that were clinical for CSS were also measured via esophageal method and were compared to the healthy manatee measurements

Results and discussion

* The esophageal measurement was significantly different (higher) from oral and nasal
  + Also consistent with core body temperatures measured in research settings with more invasive techniques
* Oral and nasal were not significantly different from each other
* The reference interval for core body temperature as measured by esophageal temperature for the healthy manatees was 35.0–35.8 C
* The esophageal temperatures of all 4 CSS manatees were below this reference range by at least 1.2 deg C
* The procedure was well tolerated by all manatees and temperatures were attained quickly and remained stable over a 2-minute period
* Note that measurements were taken after 5 minutes out of water, so could be some environmental impact (warmer air compared to the water leading to increase core temp) on temperature though none was identified
* **Takeaway:** Esophageal temperature measurements is an accurate and non-invasive way to measure core body temperature in florida manatees

**MORTALITY TRENDS IN NORTHERN SEA OTTERS (ENHYDRA LUTRIS KENYONI ) COLLECTED FROM THE COASTS OF WASHINGTON AND OREGON, USA (2002–15)**

C. LeAnn White, Emily W. Lankau, Deanna Lynch, Susan Knowles, Krysten L. Schuler, Jitender P. Dubey, Valerie I. Shearn-Bochsler, Marcos Isidoro-Ayza, and Nancy J. Thomas

Journal of Wildlife Diseases, 54(2), 2018, pp. 238–247

**Abstract:** During 2002-15 we examined the causes of mortality in a population of northern sea otters (Enhydra lutris kenyoni). **Beachcast sea otters were collected primarily from the US coast of Washington.** Although there are no permanent sea otter residents in Oregon, several beachcast otters were collected from the Oregon coast. Infectious diseases were the primary cause of death (56%) for otters we examined. Sarcocystosis was the leading infectious cause of death (54%) and was observed throughout the study period. Some infectious diseases, such as morbilliviral encephalitis and leptospirosis, were documented for a limited number of years and then not detected again despite continued testing for these pathogens in necropsied animals. Trauma was the second most common cause of death (14%) during the study period. The continued stable growth of the Washington population of otters suggests they are able to tolerate current mortality rates.

**Key Points:**

* Infectious disease: primary cause of death for 56%
* *Sarcocystis neurona* encephalitis primary infectious disease as cause of death, peak 53% in 2011
  + CA otters: seasonal peak in March-May with sporocyst shedding by opossums
  + Genotype XIII more likely to develop severe encephalitis in past studies
* *Streptococcus phocae* – bacterial septicemia, *P multocida* synergistic pathogen
  + Some disruption of skin or mucous membranes found in half the cases
* Morbilliviral encephalitis, leptospirosis, and *T gondii* uncommon
  + *Toxoplasma gondii* – 3% (other study showed 16% of California otters)
* Trauma - 14%, blunt head trauma majority
  + Shark bites a bigger issue in California SSO population
* DCM and one case of CHF identified

**Take Home:** Sarcocystis is a leading cause of mortality in Washington Northern Sea Otters

