

**FEDERAL AID
ANNUAL RESEARCH PERFORMANCE REPORT**

ALASKA DEPARTMENT OF FISH AND GAME
DIVISION OF WILDLIFE CONSERVATION
PO Box 25526
Juneau, AK 99802-5526

PROJECT TITLE: Wildlife Health and Disease Surveillance in Alaska

PRINCIPAL INVESTIGATOR: Dr. Kimberlee Beckmen

COOPERATORS: U.S. Department of Agriculture, Alaska Department of Environmental Conservation, National Marine Fisheries- National Marine Mammal Laboratory, Alaska Department of Health and Human Services, U.S. Geological Survey, U.S. Fish and Wildlife Service (FWS), Alaska Bird Observatory, and other federal, state, and local entities.

FEDERAL AID GRANT PROGRAM: Wildlife Restoration

GRANT AND SEGMENT NR: W-33-3

PROJECT NR: 18.73

WORK LOCATION: Statewide

STATE: Alaska

PERIOD: 1 July 2004–30 June 2005

I. PROGRESS ON PROJECT OBJECTIVES SINCE PROJECT INCEPTION (*Do not complete for projects only 1 year old.*)

OBJECTIVE: Document, evaluate, and monitor the incidence of diseases in free-ranging wildlife as well as the potential impacts of disease on wildlife populations in Alaska. Ensure animal welfare considerations in the capture and handling of wildlife by the division for research or management purposes.

PROGRESS: The activities under this objective are ongoing, and accomplishment of activities annually (see below) constitutes progress on the objective.

II. SUMMARY OF WORK COMPLETED ON JOBS IDENTIFIED IN ANNUAL PLAN THIS PERIOD

Job 1. Implement the Chronic Wasting Disease (CWD) Surveillance Program.
During October 2004, I gave training in necropsy technique and CWD sample collection/submission to 3 ADF&G wildlife biologists and a wildlife technician at the Moose Research Center. In January 2005, a CWD sampling and necropsy training session was presented by myself with assistance from the state veterinarian, the University of Alaska

Please note: This is a progress report and the information contained within may be further analyzed and refined

Fairbanks (UAF) veterinarian and a contract veterinary pathologist in Fairbanks. Twenty-one ADF&G biologists/technicians, as well as biologists employed by federal agencies, received specialized training in a 2-day workshop. I supervised our wildlife biologist III in Kodiak to develop brochures and posters for distribution in Kodiak and at area offices. These materials were designed to alert hunters and the public about CWD and ADF&G's surveillance plan. Tissue samples from a total of 441 free-ranging cervids (of which 394 were Sitka black-tailed deer from Kodiak) and 2 captive caribou were collected in the state by me and ADF&G personnel under my supervision. All tissues were submitted for CWD testing at Colorado State Veterinary Diagnostic Laboratory (all negative).

Job 2. Coordinate the West Nile Virus (WNV) surveillance of wild birds in Alaska.

I necropsied and collected brain samples from 18 target corvids and raptors for WNV testing. These sampling activities were summarized and reported to the Alaska WNV Surveillance Task Force. I revised the sample collection and processing protocols to be better coordinated with the Alaska State Virology Laboratory needs so the brain tissues were transferred immediately into the essential media prior to transfer to the laboratory. I trained veterinary student intern to conduct bird necropsies and sample collections from May to July 2005.

Job 3. Maintain the blood, serum and tissue banks.

I supervised a UAF student intern to conduct an inventory of the serum archive to enhance availability of serum for disease surveillance and research activities both within and outside of ADF&G. The inventory logged in over 28,000 accessions thus far. Blood samples from 1225 animals were received, processed, accessioned, and a subset sent to laboratories for analysis. An ultra-low freezer containing all the genetics samples broke down twice (but samples were saved before they thawed). The freezer was repaired but will need to be replaced.

Job 4. Conduct disease and parasite surveillance and monitor changes in disease patterns.

One hundred-seventy-three carcasses and animal parts were accessioned and examined postmortem. On every fresh carcass where spleen or liver was available, the tissues were sampled for a Tularemia testing program at UAF. Three significant wildlife die-off investigations were conducted. The first was a die-off of Dall sheep in July 2004 that was diagnosed as *Pasteurella pneumonia* and contagious foot rot. The second was a die-off of red polls that led to the isolation of an enteric pathogen, *Escherichia albertii*, that had only been previously known from a diarrhea outbreak in children in Bangladesh. The third die-off occurred in moose calves in the Interior and led to the recognition of a parasite-induced secondary bacterial peritonitis/septicemia syndrome.

I conducted a half-day workshop in Fairbanks on wildlife diseases and recognition of foreign animal diseases that was attended by 35 biologists and technicians.

A veterinary student intern and I conducted a health assessment of the Northern Alaska Peninsula caribou herd in conjunction with a joint ADF&G-FWS capture and calf mortality study. Nineteen collared calves were found dead in good postmortem condition and were necropsied. Four calves and 3 ill adult caribou were collected by gunshot from an R-44

helicopter for pathologic examination and disease surveillance. Results of the health assessment included serologic testing that detected the recent introduction of several bovine respiratory viruses and severe parasite problems within the herd. Serum from the archive was used to determine the distribution and prevalence of the coccidian parasites *Neospora* and *Toxoplasma* in 201 moose, 206 caribou, 200 wolves, 12 coyotes and 8 foxes in Region II and III. Histopathology and a new ectoparasite detection technique employed in house with the help of a student intern, led to the first detection of *Trichodectes canis*, the dog louse, on wolves north of the Alaska range. Fecal samples from 52 moose and 47 bison were collected during captures or by hunters and were accessed and submitted to federal laboratories for surveillance for Johne's Disease (*Mycobacterium avium paratuberculosis*) by fecal culture in collaboration with the state veterinarian. A fecal culture collected during capture for radiocollaring the previous fall from a moose near Aniak was reported by the National Veterinary Diagnostic Laboratory to be positive for *Mycobacterium avium paratuberculosis*. In consultation with the state veterinarian, the decision was made to collect the moose and her calf by gunshot from an R-44 helicopter in July 2004 to remove this potential disease threat and confirm the existence of pathology associated with this organism in moose. However, after the animal was killed, the federal laboratory admitted a reporting error and the animal's sample did not contain the Johne's disease organism after all. *Brucella* serosurveillance continues with no evidence of the expansion of this disease to additional caribou herds or to wildlife south of the Alaska Range. An investigation of the possible contribution of infectious disease to the declining numbers of Delta bison was conducted and effectively ruled out as the cause of the population decrease.

Job 5. Monitor levels of environmental contaminants in species of concern. Results of chlorinated hydrocarbons and brominated diphenyl ethers analysis in blubber from 20 Steller sea lions were received and interpreted. I analyzed the organochlorine contaminants data from 145 Steller sea lion samples collected by ADF&G from 1998 to 2004 and summarized the results in 2 draft manuscripts.

Job 6. Review literature, prepare annual progress reports, a final report, and manuscripts for publication in refereed literature.

A semiannual and a final annual report on CWD surveillance activities were submitted to USDA. Two manuscripts on environmental contaminants in Steller sea lions were drafted and are in review with co-authors. A preliminary report on the "Health Assessment and Calfhood Mortality in the Northern Alaska Peninsula Caribou Herd" was drafted and submitted. I attended scientific sessions and presented scientific papers in an oral and poster format at the Wildlife Disease Association conference in San Diego at the end of August and the Sea Lions of the World Conference in Anchorage at the beginning of October. I have submitted abstracts of the Biennial Conference on the Biology of Marine Mammals and the annual joint conference of the American Association of Wildlife Veterinarians and American Association of Zoo Veterinarians. I was also co-author on 5 other presentations given at scientific meetings. I am co-author on 2 publications submitted to or accepted by journals during this period. Abstracts of publications and presentations are given in the Appendix.

Job 7. Perform the duties of attending veterinarian.

I participated in 3 Steller sea lion capture trips and in the June 2005 Steller sea lion pup branding activities. One capture-related mortality occurred during anesthesia of a pup for branding. I participated in capture of moose calves for radiocollaring in the Experimental Micro-Management Area of 19D East. I examined briefly the carcasses of previously collared moose that were detected as having died in the previous month. In October 2004 I conducted the annual facility inspection of the Moose Research Center. Also in October, I participated in teaching a wildlife immobilization training workshop for wildlife biologists in collaboration with the Institute of Arctic Biology at UAF. In May 2005 I attended The Wildlife Seminar for Emergency Animal Disease Preparedness presented at the Southeast Wildlife Disease Cooperative in Athens, Ga. With that training I became the Alaska Wildlife Liaison for Animal Disease Emergencies. As chairman of the Division of Wildlife Conservation Animal Care and Use Committee, I review 18 new Assurance of Animal Care forms (IACUC [Institutional Animal Care and Use Committee] protocols) for wildlife research projects that involved the handling of live animals. I advised and dispensed immobilizing drugs for 31 wildlife capture operations conducted by the DWC.

III. ADDITIONAL FEDERAL AID-FUNDED WORK NOT DESCRIBED ABOVE THAT WAS ACCOMPLISHED ON THIS PROJECT DURING THIS SEGMENT PERIOD

IV. PUBLICATIONS (*List only publications prepared or published during this reporting period.*). Two manuscripts were prepared and are currently in the editing process with co-authors. I am co-author on 3 publications submitted to or accepted by journals during this period.

1. In Press, Journal of Wildlife Diseases: Infectious disease and the decline of Steller sea lions (*Eumetopias jubatus*) in Alaska: insights from serological data
Kathy A. Burek,^{1,11} Frances M. D. Gulland,² Gay Sheffield,³ **Kimberlee B. Beckmen**,³ Enid Keyes,⁴ Terry R. Spraker,⁵ Alvin W. Smith,⁶ Douglas E. Skilling,⁶ James F. Evermann,⁷ Jeffery L. Stott,⁸ Jerry T. Saliki⁹, Andrew W. Trites¹⁰

ABSTRACT: Serology data were examined to determine whether infectious disease may have played a role in the decline of Steller sea lions (*Eumetopias jubatus*) in the Gulf of Alaska and Aleutian Islands. Available published data, historical unpublished data, and recent collections (1997–2000) were compared and reviewed. Data was stratified by geography in order to compare the declining western Alaska population in the Aleutian Islands regions through eastern Prince William Sound to the increasing population in Southeast Alaska. Prevalences of antibodies from the 1970s to early 1990s were noted for *Leptospira interrogans*, *Chlamydophila psittaci*, *Brucella* spp., phocid herpesvirus 1, and canine parvovirus. Serum samples collected and analyzed from 1997 to 2000 were tested for antibodies to these agents as well as to caliciviruses, marine mammal morbilliviruses, and canine adenoviruses 1 and 2. Conclusions could not be drawn about changes in the prevalence of exposure to disease agents during the decline of Steller sea lions because data were not comparable either because of inconsistencies in test techniques, or because the samples were either not collected in all decades from all regions or were not tested for antibodies to the same disease agents in different decades. Despite these shortcomings, the available data contained no convincing evidence of significant exposure of Steller sea lions to morbilliviruses, *B. spp.*, canine parvovirus or *L. interrogans*. Steller sea lions have been exposed

to a phocid herpesvirus, caliciviruses, canine adenovirus, and *C. psittaci* or to cross reactive organisms in regions of both increasing and decreasing sea lion abundance. These disease agents are not likely to have been the primary cause of the decline because they are found at comparable levels in both the increasing and the decreasing populations. However, they may have contributed to the decline or impeded recovery of the Steller sea lion population due to undetected mortality and morbidity, or reduction of fecundity and body condition in animals under other stresses. Systematic monitoring for disease agents and their effects is needed to determine whether infectious disease is currently playing a role in the decline and lack of recovery of Steller sea lions.

2. Submitted to Archives of Virology: Genetic identification of novel poxviruses of cetaceans and pinnipeds. A.J. Bracht¹, R.L. Brudek¹, R.Y. Ewing², C.A. Manire³, K.A. Burek⁴, C. Rosa⁵, **K.B. Beckmen**⁶, J. E. Maruniak⁷ and C.H. Romero¹

ABSTRACT: Novel poxviruses were identified in skin lesions of several species of cetaceans and pinnipeds using polymerase chain reaction targeting *Chordopoxvirinae* DNA polymerase and DNA topoisomerase I genes. With the exception of parapoxviruses, no molecular data of marine mammal poxviruses were available to infer genetic and evolutionary relatedness to terrestrial vertebrate poxviruses. Viruses were assigned to a cetacean poxvirus 1 (CPV-1) group based on nucleotide and amino acid identities of gene fragments amplified from skin lesions of Asian bottlenose (*Tursiops aduncus*), Atlantic bottlenose (*Tursiops truncatus*), rough-toothed (*Steno bredanensis*), and striped (*Stenella coeruleoalba*) dolphins. A different poxvirus was detected in skin lesions of a bowhead whale (*Balaena mysticetus*) and provisionally assigned to a CPV-2 group. These viruses showed highest identity to terrestrial poxviruses of the genus *Orthopox* and *Suidpox*. A novel species-specific poxvirus was also identified in skin lesions of Steller sea lions (*Eumetopias jubatus*). None of these poxviruses were found to have amplifiable hemagglutinin gene sequences. Novel parapoxviruses were also identified in skin lesions of Steller sea lions and spotted seals (*Phoca largha*). A significant degree of divergence was observed in sequences of Steller sea lion parapoxviruses, while those of spotted seals and harbor seals (*Phoca vitulina*) were highly conserved.

3. Submitted to: Archives of Virology: Prion genotypes in feral herds of Alaska caribou. George M. Happ,¹ Heather J. Huson,¹ Kimberlee B. Beckmen² and Lorna J. Kennedy³

ABSTRACT: The prion genes were sequenced in three herds (Porcupine, Western Arctic, and Delta) of Alaska caribou (*Rangifer tarandus grantii*). Five single nucleotide polymorphisms were detected including one at codon 138 [serine/asparagine] which is present at similar frequencies (ca. 0.65/0.35) in all three allopatric herds, each of which is a representative subpopulation of the North America caribou metapopulation. Caribou genes are identical or nearly so to prion alleles of wapiti, white-tailed and/or mule deer, suggesting that genetics poses no barrier to the spread of Chronic Wasting Disease from middle-latitude deer to high latitude caribou.

4. In Draft: Organochlorine contaminant concentrations in blubber of free-ranging Steller sea lion (*Eumetopias jubatus*) pups and juveniles in Alaska. **Kimberlee B. Beckmen**,^{1*} Kathleen A. Burek,² Kenneth W. Pitcher,³ and Gina M. Ylitalo⁴ ABSTRACT: Blubber samples were collected by surgical biopsy and necropsy from 145 free-ranging pup and juvenile Steller sea lions (*Eumetopias jubatus*) in Alaska over 6 years (1998–2004) to assess exposure of selected

organochlorine (OC) contaminants (e.g., dioxin-like PCBs, DDTs) in the stable eastern stock in Southeast Alaska (SE) as compared to the depleted western stock in Gulf of Alaska (GOA) and eastern Aleutian Islands (EAI). Results of a rapid OC screening were used to assess exposure of selected organochlorine (OC) contaminants (e.g., dioxin-like PCBs, DDTs) in dependent pup through subadult in consideration of developmental age. Transplacental transfer of OCs was extremely low. Concentrations of OCs peaked in pups sampled between 2 weeks and 1.5 months of age and declined by midway through the suckling period and then increased again through the first year of the dependent period and through the presumed weaning period. Pesticides and brominated di-benzo furans were determined by GC/MS in 30 and 14 animals respectively, including 4 that were sampled at 5-month intervals. These data suggest that exposure to the OCs is at a level of concern, especially in young pups in portions of the range of the declining western stock of Steller sea lions.

5. In Draft: Organochlorine contaminant concentrations in multiple tissue matrices of live Steller sea lions (*Eumetopias jubatus*) in Alaska. Kimberlee B. Beckmen,^{1*} Kathleen A. Burek,² Kenneth W. Pitcher,³ and Gina M. Ylitalo⁴ ABSTRACT: Blood, blubber, milk, and feces were collected from 53 free-ranging and 3 captive Steller sea lions (*Eumetopias jubatus*) in Alaska over 6 years (1998–2003) to assess exposure of selected organochlorine (OC) contaminants (e.g., dioxin-like PCBs, DDTs) in different matrices. The relationships of various OC contaminants in multiple matrices from individuals were examined to determine suitability for exposure monitoring in live animals with decreasing invasive sampling techniques. Concentrations of some OC contaminants in blubber, milk and blood were highly correlated in individuals; however, fecal concentrations were not well correlated to any other matrix. These findings indicate that a whole blood sample may be useful as a non-invasive indicator of relative contaminant exposure in lieu of surgical blubber biopsy.

V. RECOMMENDATIONS FOR THIS PROJECT (optional)

VI. APPENDIX

1. Abstract submitted and accepted for an oral presentation for the upcoming American Association of Wildlife Veterinarians and American Association of Zoo Veterinarians joint annual conference:

An investigation of moose mortalities in Alaska by **Kimberlee Beckmen, MS, DVM, PhD*** and Kathy Burek, DVM, MS, diplomate ACVP

ABSTRACT: In 2004–2005 we undertook an investigation to determine the causes of death in moose found dead in Alaska. Intact moose carcasses that were found by department personnel or reported by the public that were not obviously due to human-induced trauma, underwent detailed post mortem examinations and histopathology. Additionally, moose with radiocollars that were detected in mortality mode were investigated when predation was not the proximate cause of death. Fifty-four moose were examined and a variety of diseases or parasites, some not previously reported in Alaskan moose, were discovered. A late winter cluster of mortalities, mostly in calves, had gross and histologic lesions of vasculitis and fibrinopurulent peritonitis (diagnostics pending). Other notable diagnoses include: meningoencephalitis, peracute Clostridial septicemia, metastatic malignant melanoma, mesothelioma, meningoencephalitis,

fungal pneumonia, and pyometra with uterine rupture, severe pathology associated with rumen flukes in debilitated moose, copper deficiencies, degenerative myopathy/granulocytic myositis, granulomatous steatitis and perineuritis.

2. Co-authored abstract prepared and submitted for the upcoming Biennial Conference on the Biology of Marine Mammals: Expression of the capsid gene of new genotypes of marine caliciviruses isolated from Steller sea lions (*Eumetopias jubatus*) from Alaska

McClenahan, Shasta D.¹, Burek, Kathy A.², **Beckmen, Kimberlee B.**³, Knowles, Nick J.⁴, and Romero, Carlos H.

ABSTRACT: Marine caliciviruses, exemplified by San Miguel sea lion viruses (SMSV), are a group of single-stranded, non-enveloped, icosahedral, positive-sense RNA viruses of ocean origin, belonging to the genus Vesivirus of the *Caliciviridae*. The first isolation of an SMSV (SMSV-1) was from a California sea lion in 1972. Since then, at least 17 serotypes of SMSV have been identified, most from pinnipeds, and one from a cetacean. Currently, identification of new caliciviruses is based primarily on serological methods; however, due to high mutation rates, emerging marine caliciviruses are frequently not typeable. Identification of new marine caliciviruses, therefore, requires molecular characterization of genotypes and the production of new antigens for serology to represent the viruses currently circulating in the population. Two new isolates of marine calicivirus, from blister fluids and oral and rectal swabs from Steller sea lions (*Eumetopias jubatus*) in southeastern Alaska, have been identified by RT-PCR and sequencing of the complete capsid protein gene. Multiple sequence analyses and phylogeny of the amino acid sequences deduced from both capsid proteins and their homologues from other marine caliciviruses available in the GenBank database indicate that these viruses correspond to new marine calicivirus genotypes. To generate antigens representing these genotypes, the full capsid gene of both isolates was cloned into a baculovirus expression vector, pBlueBac4.5, and co-transfected into Sf-21 insect (*Spodoptera frugiperda*) cell cultures with wild type baculovirus to generate recombinant baculoviruses expressing the full capsid protein of each genotype. These antigens are being used in the development of ELISAs for sero-epidemiological surveys to detect calicivirus antibodies specific for these new genotypes.

3. Co-authored abstract presented by the lead author at the International Association of Aquatic Animal Medicine Conference in Seward AK, May 2005: New genotypes of marine caliciviruses isolated from Steller sea lions (*Eumetopias jubatus*) from Alaska

Shasta D. McClenaha,*Carlos H. Romero, Kathy A. Burek, **Kimberlee B. Beckmen**, Nick J. Knowles

ABSTRACT: Marine caliciviruses as exemplified by San Miguel sea lion viruses (SMSV) are a group of single stranded, nonenveloped, icosahedral, positive-sense RNA viruses of ocean origin belonging to the genus Vesivirus within the *Caliciviridae*. The first isolation of a SMSV was in 1972 from a pinniped. This virus was named SMSV-1 and since then, at least 17 serotypes of SMSV have been identified. Blister fluids and oral and rectal swabs were harvested from two declining populations of Steller sea lions (*Eumetopias jubatus*) in southeast Alaska. Samples were inoculated onto African green monkey kidney cells (Vero) and Madin-Darby canine kidney cells (MDCK) resulting in the isolation of several caliciviruses identified by RT-PCR and sequencing of an approximately 700-bp fragment within the capsid protein gene. Two of the

isolates were randomly selected for further characterization. Isolate V810 was recovered from an oral swab harvested in 2002 from an apparently healthy animal while isolate V1415 originated from a flipper vesicle fluid harvested in 2004 from a young animal. Isolate V810 grew well in Vero and MDCK cultures while isolate V1415 only grew in MDCK cells. Total RNA was extracted from the infected cell monolayers and reversed transcribed to produce cDNA. Primers specific for the capsid gene of marine caliciviruses were used to amplify the full capsids from the cDNA using PCR. Both capsids were approximately 2 kbp in length, consistent with other calicivirus capsids' size. The PCR fragments were cloned into pCR2.1-TOPO T/A and sequenced. The capsid gene from isolate V1415 was 2166-bp in length while that of V810 was 2157-bp. Pair-wise alignments of the full capsid genes showed that they were 79.6 % and 88.4 % identical at the nucleotide and amino acid levels, respectively. Multiple sequence analyses and phylogeny of the amino acid sequences deduced from both full capsids and their homologues from other marine caliciviruses available in the GenBank database revealed that these isolates constituted new marine calicivirus genotypes. Sera from Steller sea lions older than 14 weeks-of-age generally had antibody titers greater than 128 against the recent local isolate V1415. On the other hand, sera from animals about 2-months-old had antibody titers that varied greatly between <4 and >8192, most likely due to waning of maternal antibodies or early calicivirus infection. The capsid gene of isolate V1415 was subcloned into the baculovirus expression vector pFastBac and transfected into Sf-21 (*Spodoptera frugiperda*) insect cell cultures to generate a recombinant baculovirus that expresses the full capsid protein. This antigen is being used in the development of an ELISA for the detection of calicivirus antibodies.

4. Authored abstract prepared and submitted for the upcoming Biennial Conference on the Biology of Marine Mammals: Hookworms in Steller sea lions (*Eumetopias jubatus*) in Alaska **Beckmen, Kimberlee B.**^{1*}; Burek, Kathy A.²; Gelatt, Tom³ Morado, Frank⁴; Nadler, Steve⁵; Lyons, Eugene T.⁶ ABSTRACT: Recent disease surveys in Steller sea lions (SSLs) in Alaska have detected the presence of a hookworm similar to *Uncinaria spp.* described in other otariids. Our objectives were to determine hookworm prevalence and loads, pathologic effects and to identify the hookworm species in SSLs. Fecal sedimentations were performed on animals 2–35 months old (n=197). Hookworm eggs were not seen after 5 months and 2–3 month olds had a prevalence of 69% overall. Fecal egg counts were done in 2–4 week old (n=77) and 2 month old (n=21) pups in Southeast Alaska. Counts ranged from 0 to 4950 eggs/gm in 2–4 week olds and 53–889 eggs/gm of formalin-fixed feces in 2 month olds. Percent patent infections were 55% and 76% respectively in the 2–4 week olds and 2 month olds. Eggs counts and hematocrit were negatively correlated in 2–4 wk old pups, while there was no correlation in the 2 month olds. All of 14 dead 2–4 week old pups were positive for hookworm adults. Total intestinal worm burdens ranged from 18 to 3477. Presumptive hookworm related lesions included gastrointestinal hemorrhage, organ pallor, and migration tracts in the liver. These parasites are morphologically identified as an *Uncinaria sp.* Molecular studies on 20 worms have indicated the sequenced products are identical to *U. lucasi*. Preliminary work has been done to study the life cycle of this parasite. Development to the free-living L3 occurs within the egg, similar to what occurs in northern fur seal (*Callorhinus ursinus*) and California sea lion (*Zalophus californianus*) hookworms. Parasitic L3 have been recovered from the ventral abdominal blubber from pups. Further studies on hookworm-associated pathology are critical considering that parasite loads encountered are well above those associated with significant mortalities in other pinniped species, and that we are

missing comparative data from the declining western stock.

5. Co-authored abstract presented by the lead author at the America Association of Laboratory Diagnosticians: Mortality in Redpoll finches in Alaska (*Carduelis flammea*) due to *Escherichia albertii* J.L. Oaks¹, **K.B. Beckmen**², T.E. Besser¹, G.H. Haldorson¹, D.S. Bradway¹, F.R. Rurangirwa¹, and K.A. Burek.³

ABSTRACT: A mass mortality event affecting Common Redpolls (*Carduelis flammea*) in Alaska was associated with infection by the bacterium *Escherichia albertii*. Reports of dead Redpolls at back-yard feeders began in late December 2004. The period of highest mortality (one to six dead birds per feeder) coincided with a period of prolonged sub-minus 40°C temperatures in late January, although daily reports continued into early February. Dead bird reports ceased suddenly, with the last on February 24th. The local at-risk population at the beginning of the outbreak was about 8000 birds, which was a historic high for the area. Approximately 100 deaths were documented, although it is assumed that the actual death toll was considerably higher.

Gross post-mortem lesions were not consistently seen. One culture-positive bird had fecal matter pasted around the vent and intestines that were dark and distended with excessive yellow to green digesta. All culture positive birds had adequate pectoral muscle mass, suggesting acute death. Histologic lesions consisted mainly of mild enteritis. Although there were no anatomic changes to indicate septicemia, *E. albertii* was isolated in high numbers and in pure cultures from the intestines and tissues of five Redpolls that died during the peak of the epornitic. These five cases were from a 132 km diameter region around Fairbanks, AK. All of the *E. albertii* isolates were indistinguishable by pulsed-field gel electrophoresis. Other birds, including Redpolls, a Black-capped chickadee (*Poecile atricapilla*), a Crossbill (*Loxia curvirostra*), and a Boreal owl (*Aegolius funereus*) that were known to have died of other causes were cultured as controls and were negative for *E. albertii*. A Redpoll mortality event occurring at the same time in the Juneau, AK area was associated with isolation of *Salmonella enterica* serovar Typhimurium (but not *E. albertii*) from feces and tissues, and exhibited lesions typical of severe *Salmonella* enteritis.

E. albertii is a recently described member of the *Enterobacteriaceae* that has been associated with diarrheal illness in humans in Bangladesh, but has not been previously associated with disease or infection in animals. The *E. albertii* isolated from these Redpolls is very similar to the description of an atypical *E. coli* previously reported from a finch mortality event in Scotland. Our identification of *E. albertii* was established by 16S rDNA gene sequence and by phenotypic similarity to previously reported *E. albertii* isolates. The Redpoll isolates exhibit several biochemical reactions atypical for *E. coli*, including lack of lactose fermentation. The isolates also exhibit some biochemical reactions atypical of previously reported *E. albertii* isolates, including production of indole from tryptophan. PCR and DNA sequencing were used to confirm the presence of genes for intimin and for cytolethal distending toxin; the DNA sequences of these proteins differed significantly from those previously reported from *E. albertii* and the Scottish finch isolates, and were more similar to genes previously reported from *E. coli*.

6. Invited abstract submitted to the American Chemical Society meeting in Fairbanks:
Organochlorine contaminant concentrations in multiple tissue matrices of Steller sea lions
(*Eumetopias jubatus*) in Alaska

Kimberlee B. Beckmen,^{1*} Kathleen A. Burek,² Kenneth W. Pitcher,³ Brian Fadely⁴, and Gina M. Ylitalo.

ABSTRACT: Blubber samples were collected by surgical biopsy and necropsy from 145 free-ranging pup and juvenile Steller sea lions (*Eumetopias jubatus*) in Alaska over 6 years (1998–2004) to assess exposure of selected organochlorine (OC) contaminants (e.g., dioxin-like PCBs, DDTs) in the stable eastern stock in Southeast Alaska (SE) as compared to the depleted western stock in Gulf of Alaska (GOA) and eastern Aleutian Islands (EAI). Results of a rapid OC screening were used to assess exposure of selected organochlorine (OC) contaminants (e.g., dioxin-like PCBs, DDTs) in dependent pup through subadult in consideration of developmental age. Transplacental transfer of OCs was extremely low. Concentrations of OCs peaked in pups sampled between 2 weeks and 1.5 months of age and declined by midway through the suckling period and then increased again through the first year of the dependent period and though the presumed weaning period. Pesticides and brominated di-benzo furans were determined by GC/MS in 30 and 14 animals respectively, including 4 that were sampled at 5-month intervals. These data are compared to northern fur seals (*Callorhinus ursinus*) and suggest that exposure to the OCs is at a level of concern especially in young pups in portions of the range of Alaskan Steller sea lions. Blood, milk, and feces were collected from 53 of the animals undergoing blubber biopsy to assess exposure of selected OC contaminants in different matrices. The relationships of various OC contaminants in multiple matrices from individuals were examined to determine suitability for exposure monitoring in live animals with decreasing invasive sampling techniques. Concentrations of some OC contaminants in blubber, milk and blood were highly correlated in individuals; however, fecal concentrations were not well correlated to any other matrix. These findings indicate that a whole blood sample may be useful as a non-invasive indicator of relative contaminant exposure in lieu of surgical blubber biopsy.

7. Co-authored abstract given as an oral presentation by the lead author at the Sea Lions of the World conference: Hookworms in Steller sea lions (*Eumetopias jubatus*) in Alaska

Kathy A. Burek,^{1*} **Kimberlee B. Beckmen**² Tom Gelatt,³ Frank Morado,⁴ Steve Nadler.⁵

ABSTRACT: Recent disease surveys in Steller sea lions (SSLs) have detected the presence of a hookworm that is similar to *Hncinaria spp.* Described in other otariids. Pathologic findings in previously described pinniped hookworm infestations occur in young pups and include anemia, intestinal hemorrhage and inflammation, skin lesions, intestinal perforations and peritonitis. Our objectives were to determine hookworm prevalence and loads, pathologic effects and to identify the species in SSLs in southeast Alaska.

Fecal sedimentations were performed on animals 2–35 months old (n = 126). No trends were noted by region. Hookworm eggs were not seen in animals older than 5 months and 2–3 mo old animals had a hookworm prevalence of 69%. Fecal egg counts in 2–4 wk old animals (n = 38) ranged from 0 to 540 eggs/gm of formalin-fixed feces. Percent patent infections were similar between areas at 50% (n = 26) and 41% (n = 12). Pups with patent infections had lower hematocrits (n = 24) (r = -0.427, p=0.04). All of the 2–4 wk old dead pups (n = 24) were positive for hookworm adults. Total intestinal worm burdens ranged from 18 to 3477. Presumptive

hookworm related lesions in these animals included gastrointestinal hemorrhage, organ pallor, and migration tracts in the liver. These parasites have been identified morphologically as an *Uncinaria sp.* Preliminary genetic data on a small sample (2) have indicated the sequenced products are identical to *U. lucasi*. Further studies on hookworm associated pathology are critical considering that parasite loads we have encountered are well above those associated with significant mortalities in other pinniped species, and that we are missing data from the declining western stock.

8. Abstract and oral presentation given at the Wildlife Disease Association meeting in San Diego: A health assessment approach to Steller Sea Lion research in Alaska

Kimberlee B. Beckmen MS, DVM, Ph.D,^{1*} Kathy A. Burek, DVM, MS, Dipl. ACVP,² Lorrie D. Rea MS, PhD,³ Thomas S. Gelatt MS, PhD.³

ABSTRACT: Utilizing collaborative research opportunities with several agencies, data has been collected on over 400 SSL pups and juveniles during live-capture/release. Morphometrics and foraging studies examine differences between sexes, ages, regions, stocks, etc. Health and disease information is collected concurrently on the same individuals as well as pathological examinations on dead animals. Samples on each live-captured animal are collected for hematology, serology, clinical chemistries, organochlorine and heavy metal analysis, histopathology of lesions, bacteriology (culture and PCR), virology (culture and PCR), mycology, parasitology and on some animals for more extensive immune function studies. Selected disease agents currently under surveillance by culture, PCR, or serology include: *Chlamydophila psittaci*, poxviruses, caliciviruses, phocid herpesvirus-1, *Toxoplasma gondii*, morbilliviruses, *Leptospira interrogans*, *Salmonella sp.*, pathogenic *E coli*, influenza A, *Brucella spp.*, canine parvovirus, canine adenovirus 1 + 2, *Sarcocystis neurona*, *Uncinaria sp.*

9. Authored abstract given as an oral presentation at the Sea Lions of the World conference A health assessment approach to Steller sea lion research in Alaska

Kimberlee B. Beckmen MS, DVM, Ph.D,^{1*} Kathy A. Burek, DVM, MS, Dipl. ACVP,² Lorrie D. Rea MS, PhD,³ Thomas S. Gelatt MS, PhD.³

ABSTRACT: Data has been collected on over 400 SSL pups and juveniles during live-capture/release. Morphometrics and foraging studies examine differences between sexes, ages, regions, stocks, etc. Health and disease information is collected concurrently on the same individuals. Samples on each animal are collected for hematology, serology, clinical chemistries, organochlorine (OC) and heavy metal analysis, histopathology of lesions, microbiology, parasitology and on some animals for more extensive immune function studies. There has been no evidence of exposure to known marine mammal epidemic disease agents including morbilliviruses, *Leptospira interrogans*, Influenza A and *Brucella sp.* Some disease agents were demonstrated by serology +/- culture and PCR to be endemic, including *Chlamydophila sp.*, Poxvirus, Caliciviruses, Phocid herpesvirus-1, *Toxoplasma gondii*, Canine adenovirus, and *Sarcocystis neurona*. *Salmonella sp.* and pathogenic *E coli*. OC levels vary by region and are in some areas at levels of concern.

10. Co-authored abstract given as a student poster presentation under my supervision at the Wildlife Society Meeting: Hughes, J. Letty, and **Kimberlee Beckmen**. Hookworm parasites of the Steller sea lion, *Eumetopias jubatus*: species identity, prevalence, and population health effects

ABSTRACT: The Steller sea lion, *Eumetopias jubatus*, was listed as threatened throughout its range in 1990. Due to a continued decline, the western stock was listed as endangered in 1997. Evidence suggests that the key reason for the decline is poor recruitment or low survival rates of pups or juveniles. New research has revealed the presence of a parasitic hookworm (*Uncinaria* sp.) that may affect health and survival of the Steller sea lion population. Hookworm infestations are known to produce mortalities or affect host fitness in the northern fur seal, *Callorhinus ursinus*. Anemia is highly correlated with parasites in fur seal pups, reducing the pups' ability to oxygenate, and ulcers of the intestine may be sources of lethal bacteremias. It has been speculated that *Uncinaria* may produce similar effect in Steller sea lion pups as in northern fur seal pups. We examined the correlation of parasite density with individual pup health parameters, such as hematology, and examined the distribution range and population dynamics of the parasite. Results of this study may lead to directed efforts at improving Steller sea lion pup health and survival and help with a more effective recovery plan for the population.

11. Abstract and poster presentation given at the Wildlife Disease Association meeting in San Diego: Anesthesia, mortalities, and logistics of capture with translocation of large numbers of bears in the Alaskan Bush. **Kimberlee B. Beckmen, MS, DVM, PhD,*** Toby Boudreau, MS, Shelly Szepanski, MS, Mark Keech, MS. **ABSTRACT:** During a 3-week period in May 2003, 86 black bears (BB) and 9 grizzly bears (GB) were immobilized with Telazol® (lypholyzed tiletamine HCl/zolazepam HCl reconstituted with sterile water to 200 mg/ml) at a dose range of 1–13 mg/kg (BB) and 1–3 mg/kg (GB) with a mean induction dose of 2.7 mg/kg. The majority (n = 74) were darted from an R-44 helicopter, and the remainder were darted from the ground while restrained in foot snares or attempting to escape by climbing trees (yearling BB only). All initial immobilizations (except hand captured GB cubs) were obtained via Palmer Cap-Chur darts from a CO₂ pistol with target areas generally in the rump or shoulder musculature. Darted bears ranged in weight from 8–145 kg (BB) and 75–320 kg (GB). Bears were moved from the initial capture site to a pre-translocation processing station by one of 3 methods in order of frequency: in a sling under the helicopter, inside the helicopter, or by riverboat.

The purpose of the capture operation was to experimentally remove ursine predators of newborn moose calves in a 1368 square km area of the Kuskokwim River drainage near the Alaska Native village of McGrath. Prior to translocation, bears were either maintained under anesthesia for the duration or were allowed to recover in cages and then induced with Telazol at 2–3 mg/kg via pole syringe. Induction doses of Telazol® generally gave full-restraint for 40 minutes before any additional drugs were required. For maintenance of anesthesia prior or during transport, bears were given one dose of 1 mg/kg of Telazol® (IM by hand injection) when purposeful movement was noted. This second dose usually gave an additional 40 to 60 minutes of adequate restraint. Thereafter, if additional restraint was required during transport, bears were given hand injections of 10–25 mg diazepam and 50 to 1500 mg of ketamine HCl IM. Duration of the diazepam/ketamine restraint was 20 to 30 minutes before re-dosing was required. No additional anesthesia was given within 20 minutes of reaching release sites. Of the bears

captured, 74 BB and all GB were translocated 290 to 400 km in a DeHavilland Beaver (3 to 7 bears at a time with an attendant) or singly other fixed wing aircraft.

Anesthetic complications included severe hypothermia, hyperthermia, prolonged recovery time BB at Telazol doses over 7 mg/kg, hypersalivation, petit mal seizures (more frequent in GB), and mortalities. Direct capture mortalities occurred in only 3 of 95 captures. One BB died 1 hour after darting from myocardial hemorrhage secondary to blunt chest trauma during darting. One yearling BB was euthanized because a dart wound penetrated the abdomen. Necropsy revealed a lacerated spleen and fractured femur when double darted while treed. Subsequently capture of yearling BB was immediately suspended. In addition, an adult male BB died in captivity 3 days post-transport from aspiration pneumonia (1 of only 3 bears that received xylazine 0.22 mg/kg). An adult female BB was euthanized 24 hours post-darting in captivity and was found to have significant perirenal hemorrhage that may have eventually resulted in morbidity or mortality. Many bears were able to lift their heads at the time of departure of the transport crew. Three bears were able to rise and walk away before crew departure. No bears that were released failed to recover from anesthesia or leave the release area. Twenty-two BB received radio-collars for tracking purposes and of those, 1 BB died during the summer of unknown causes. All bears were marked with ear tags and colored flags indicating the withdrawal time for meat consumption. Only two BB were taken by hunters and both occurred after the withdrawal time.

VII. PROJECT COSTS FOR THIS SEGMENT PERIOD

Stewardship Investment items purchased: *list any equipment or other items purchased for which the cost of the individual item was \$5,000 or more (include cost)*

None

Total Costs

FEDERAL AID SHARE \$64,627.5 STATE SHARE \$21,542.5 = TOTAL \$86,170

VIII. PREPARED BY:

Kimberlee B. Beckmen
Wildlife Veterinarian

SUBMITTED BY:

Mark McNay
Research Coordinator