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Wildlife health and disease surveillance in Alaska

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Research Annual Performance Report 1 July 2007–30 June 2008 Federal Aid in Wildlife Restoration W-33 Study 18.74

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PROJECT TITLE: Wildlife Health and Disease Surveillance in Alaska

PRINCIPAL INVESTIGATOR: Kimberlee Beckmen

COOPERATORS: US Department of Agriculture, Alaska Department of Environmental Conservation, University of Alaska Fairbanks, National Marine Fisheries, National Marine Mammal Laboratory, Alaska Department of Health and Human Services, US Fish and Wildlife Service, The North Slope Borough, University of California Davis and the University of Tennessee

FEDERAL AID GRANT PROGRAM: Wildlife Restoration

GRANT AND SEGMENT NO. W-33

PROJECT NO. 18.74

WORK LOCATION: Alaska, Statewide

STATE: Alaska

PERIOD: 1 July 2007-30 June 2008

I. PROGRESS ON PROJECT OBJECTIVES SINCE PROJECT INCEPTION

The <u>Wildlife Health and Disease Surveillance in Alaska</u> project is ongoing. The project statement was revised for the period July 1, 2007 to June 30, 2012. The overall objectives for the project are to:

- 1) 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.
- 2) Ensure animal welfare considerations in the capture and handling of wildlife by the Division for research or management purposes.

Detection of the introduction of new diseases or parasites, expansion of geographic or host range, and the presence of zoonotic diseases all can have management implications. Continued intense surveillance for introduction of Chronic Wasting Disease, Moose Winter Tick, Johnes Disease, West Nile Virus, Mycoplasma pneumonia of sheep, brain worm, highly pathologic avian influenza are most critical. The routine examination of found dead wildlife and identification of parasites are crucial to these efforts. Since the institution of our CWD surveillance program, we can be highly confident at this time that we do not have CWD present in Sitka black-tailed deer in the Kodiak Archipelago. However, continued surveillance must be maintained because of the threat of captive elk on Kodiak that are recently imported and of unknown status. Other populations of wild cervids have not reached sampling levels sufficient to have confidence in our negative results. If CWD were to be detected, it would necessitate immediate, wide scale management actions by the department and plans for these scenarios must be planned for. Likewise, the detection of any of the other diseases mentioned above would likely necessitate quick and decisive management actions by the department.

The disease surveillance program has lead to the detection of the expansion of the range of the biting dog louse on wolves north of the Alaska range. This has lead to a preliminary and on-going study of a new method for remote delivery of oral treatment to mitigate the effects on wolves. This method appears to be more effective and much less expensive to administer than previous attempts at lice eradication. If this study proves the method to be efficacious, it may be considered as a management tool to increase pelt quality of wolves in lice infected packs and thus maintain or enhance trapping effort in these areas.

The health assessment of Northern Peninsula Caribou Herd revealed severe parasite and nutritional problems as well as overwhelming neonatal calf mortality due to predation. A field study to examine the effect of treatment of parasite burden with ivermectin (removing the small stomach worm, *Ostertagia*) may provide information on the efficacy of treatment to boost calf production, survival and herd health. The decline in this population is so severe; all hunting including subsistence was halted. Without drastic measures, it appears this population may decline to unrecoverable levels. Understanding the role that this parasite plays in the health and survival of caribou may be key to mitigating the decline in conjunction with management of predation.

The studies of trace mineral status and disease/parasites of Dall's sheep and muskoxen are in preliminary stages. However, these studies will be important to determine if nutritional, infectious diseases or parasites are contributors to population declines or reproductive suppression. Depending on the responsible factors, management actions to supplement, treat or prevent spread of disease may be viable management tools for these populations. First and foremost, the significance of the effects of these factors on population health must be investigated.

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

JOB/ACTIVITY 1: Maintain the Chronic Wasting Disease Surveillance Program.

I again successfully obtained funding from the USDA for the DWC work plan and surveillance activities. I supervised one full-time employee conducting the CWD program.

During our FY08 (federal fiscal year 07) of our CWD surveillance program, we've tested tissues from hunter harvested animals as follows: 178 Sitka Black-tailed deer and 15 elk. In addition, we conducted targeted surveillance testing on 7 SBT deer, 4 moose and 10 caribou. All have been negative for CWD by the gold standard test, immunohistochemistry of the obex, retropharyngeal lymph node and tonsil.

I attended the CWD Symposium at the Wildlife Disease Association Meeting in Colorado in August.

Federal funds were used to pay salaries, supplies and services on this task.

JOB/ACTIVITY 2: Maintain the blood, serum and tissue banks.

Serum from 143 and blood from 96 samples were accessioned into the archive from mammals that are captured by ADFG personnel. Blood, serum or tissues as suitable were also collected at necropsy on 153 specimens presented for postmortem examination. Samples were accessed to outside investigators and graduate students, including the University of Alaska Fairbanks Museum, University of Tennessee, University of California – Davis, California Department of Fish and Game, who are working on collaborative projects with ADFG. One ultra cold freezer broke down and was unrepairable so was replaced; samples were redistributed before thawing occurred.

Federal funds were used to pay salaries, supplies and services on this task.

JOB/ACTIVITY 3: <u>Conduct disease and parasite surveillance and monitor changes in</u> <u>disease patterns</u>.

Tissues, parasites, or whole carcasses presented by the public, as well as incidental takes such as road-kill, capture mortalities of other investigators, and animals found dead were examined. Accessions exceeded 153 specimens. Gross diagnoses were assigned when possible and parasite identification or histopathological diagnoses were pursued on unusual cases or those with lesions of concern. Histopathological reports were received on 67 cases. A summary report on new parasite host, range, and identification was received from USDA collaborator Dr. Eric Hoberg and is attached [Amount A]. A report on serologic patterns of respiratory viral complex was generated by Biometrician Dr. Camilla Lieske (whom I supervised during the period) and is attached [Appendix B]. Dr. Lieske also developed the Steller Sealion Health Assessment and drafted a publication ready for submission [Abstract 1]. Two publications of a new parasite species discoveries, one on a nematode in caribou and moose, the other on a protozoan in brown bears, were published [Appendix C and D], and a publication on a pathologic finding in caribou was accepted for publication [Abstract 2].

A base-line health assessment of the Western Arctic Caribou was conducted. Ten caribou were collected for in depth health and disease surveillance. A collaboration with UAF faculty (Dr. Todd O'Hara) and graduate students (Cassandra Kirk and Katrina Knott) on a morbillivirus outbreak in foxes resulted in an abstract [Abstract 3] submitted to an upcoming Sept 2008 presentation at an Ecohealth Forum in Anchorage.

A collaboration with an MPVM student, Sebastian Carrasco, at the University of California Davis on bacterial flora patterns in Steller sea lions resulted in a thesis and draft manuscript [Abstract 4]. A collaboration with a vet student, Erica Stieve, at the University of Tennessee resulted in a manuscript on Toxoplasma and Neospora serologic patterns in moose, caribou, deer, wolves and coyotes which has been submitted and is in review for publication [Abstract 5]. A collaboration with a PhD student at the University of Florida, Shasta McClanahan, resulted in a dissertation, multiple meeting presentations and a manuscript accepted for publication [Abstracts 6, 14, 15, 16].

A collaboration with another MPVM student, Josephine Afema, at UC Davis resulted in a draft these on a epidemiologic investigation of the decline of the Eastern North Slope muskoxen population.

Bison serum and feces were collected from the Farewell Bison herd for Johnes Disease and Brucella tested at the request of and conducted by the Alaska Department of Environmental Conservation Office of the State Veterinarian. Brain tissues from 3 foxes and 4 wolves were submitted to the DHSS, Division of Epidemiology for rabies testing. One wolf and 1 fox were determined to be positive for rabies.

Federal funds were used to pay salaries, supplies and services on this task.

JOB/ACTIVITY 4: Monitor levels of environment contaminants in species of concern.

A collaboration with UAF faculty, Dr. Todd O'Hara, has initiated a study of mercury contamination in Steller sea lions. Hair, blood, liver, kidney and bone were collected for metals analysis on Dall's sheep, moose, muskoxen and caribou. A collaborative study on mercury in Steller sealions was published [Appendix E]. A former summer intern, Cristina Hansen, a veterinary student at the University of Illinois, completed a study on an enzyme in caribou blood that is affected by organochlorine pesticide exposure. She one the student research award from the American Board of Veterinary Toxicologists and submitted a manuscript for publication [Abstract 7].

Federal funds were used to pay salaries, supplies and diagnostic services on this task.

JOB/ACTIVITY 5: Assess the nutritional trace mineral status of Dall sheep, moose and caribou.

Blood, serum and tissues samples were collected and submitted for analysis at UAF and Wyoming State Veterinary Laboratory. A preliminary report on the caribou was drafted and a meeting with Region II collaborators was attended in Anchorage. Preliminary results on the Dall's sheep were presented as a poster [Abstract #8] at the Wildlife Disease Association annual meeting which generated additional collaborations and suggestions for further research. A report on the Nelchina moose TM status was generated and submitted to Bruce Dale. Moose calves at the Moose Research Center suffered clinical TM deficiencies so additional samples from moose calves were collected and need to be analyzed. The trace minerals studies were expanded to include muskoxen and data analyzed. Presentations of the results were given in Aug 2008 (not in the time scope of this report).

Federal funds were used to pay salaries, supplies and services on this task.

JOB/ACTIVITY 6: Review literature; prepare annual progress reports, a final report, and manuscripts for publication in refereed literature

Progress reports were generated for Federal Aid and CWD Surveillance program as well as periodic reports on disease surveillance activities. Four manuscripts were published, two have been accepted for publication, three have been submitted and are in review, two additional have been drafted [Abstracts #9 and #10]:

PUBLISHED Canadian Journal of Zoology. (2007) 85:1143-1156 [PDF Appendix C]: Serendipitous discovery of a novel protostrongylid (Nematoda: Metastrongyloidea) in caribou (*Rangifer tarandus*), muskoxen (*Ovibos moschatus*) and moose (*Alces americanus*) from high latitudes of North America based on DNA sequence comparisons. Authors: Kutz, S.J., I. Asmundsson, E. P. Hoberg, G.D. Appleyard, E.J. Jenkins, K. Beckmen, M.. Branigan, L. Butler, N.B. Chilton, D. Cooley, B. Elkin, F. Huby-Chilton, D. Johnson, A. Kuchboev, J. Nagy, M. Oakley, R. Popko, A. Scheer, M. Simard, A. Veitch. Abstract: Many protostrongylid nematode species produce dorsal spined larvae (DSL) that are shed in feces of wild ungulates. Definitive identification of DSL is rarely possible through comparative morphology and often, fecal samples are the only feasible means to assess the distribution of these nematode parasites in wildlife. In the present study, molecular techniques were employed to differentiate among protostrongylid species using DNA from individual larvae obtained in geographically extensive surveys. Partial sequences from the second internal transcribed spacer region (ITS-2) of the nuclear ribosomal DNA were used to differentiate DSL recovered from feces of caribou (Rangifer tarandus tarandus, R. t. caribou, R. t. grantii (Linnaeus, 1758)), muskoxen (Ovibos moschatus moschatus and O. m. wardi (Zimmerman, 1780)), and moose (Alces americanus gigas) in the North American Arctic and Subarctic. A previously uncharacterized and genetically distinct species was recognized based on the ITS-2 sequences of 37 DSL from 19 ungulate hosts across a range extending from Alaska to Labrador and 1 third stage larva from a slug (Deroceras laeve) collected in the Mackenzie Mountains, Northwest Territories. Sequence similarity among individuals of this putative species was 91-100%. For many individual DSL, paralogues of ITS-2 were detected. The ITS-2 sequences from this putative species were 72-77% similar to those of Varestrongylus alpenae, 58-61% similar to those of elaphostrongylines (Elaphostrongylus spp. and Parelaphostrongylus spp.), and 51-60% similar to those of other protostrongylids known in North American and some Eurasian ungulates. The sequence results indicate a discrete lineage of a currently undescribed protostrongylid, infecting muskoxen, caribou and moose across northern North America. Sympatric infections with P. andersoni were demonstrated in two caribou herds.

PUBLISHED Acta Parasitologica, 2007, 52(4), 299–304 [PDF Appendix D]; Sarcocystis arctosi sp. nov. (Apicomplexa, Sarcocystidae) from the brown bear (Ursus arctos), and its genetic similarity to schizonts of Sarcocystis canis-like parasite associated with fatal hepatitis in polar bears (Ursus maritimus). Authors: J.P. Dubey, Benjamin M. Rosenthal1, Natarajan Sundar1, G.V. Velmurugan1 and Kimberlee B. Beckmen. Abstract: The tissues of herbivores are commonly infected with cysts of parasites belonging to the apicomplexan genus *Sarcocystis*, but such sarcocysts are rare in bears. Here, we describe a new species, Sarcocystis arctosi, based on the mature sarcocysts identified in two brown bears (Ursus arctos) from Alaska, USA. Microscopic sarcocysts $(37.75 \times 20.42 \,\mu\text{m})$ had thin walls (<1 μ m). The outer layer of the sarcocyst, the parasitophorous vacuolar membrane (pvm), was wavy in outline and had minute undulations that did not invaginate towards the sarcocyst interior; these undulations occurred at irregular intervals and measured up to 100 nm in length and up to 60 nm width. The ground substance layer beneath the pvm was smooth and lacked microtubules. Longitudinally cut bradyzoites measured $5.6.6.8 \times 0.7.1.8 \,\mu$ m. A major portion of nuclear small subunit rDNA sequence obtained from these sarcocysts was similar to that previously obtained from the hepatic schizonts of a S. canis-like parasite from polar bears (Ursus maritimus).

PUBLISHED Marine Pollution Bulletin (2008) 56:1416-1421 [PDF Appendix E]: **Metal Tissue Levels in Steller Sea Lion** (*Eumetopias jubatus*) **Pups.** Authors: Amie L. Holmes, Sandra S. Wise, Caroline E. C. Goertz, J. Lawrence Dunn, Frances M. D. Gulland, Tom Gelatt, **Kimberlee B. Beckmen**, Kathy Burek, Shannon Atkinson, Mary Bozza, Robert Taylor, Tongzhang Zheng, Yawei Zhang, AbouEl-Makarim Aboueissa, John Pierce Wise, Sr. Abstract: The endangered Western population of the Steller sea lion declined for three decades for uncertain reasons. We present baseline data of metal concentrations in pups as a first step towards investigating the potential threat of developmental exposures to contaminants. Seven metals were investigated: arsenic, cadmium, silver, aluminum, mercury, lead and vanadium. Vanadium was detected in only a single blubber sample. Mercury appears to be the most toxicologically significant metal with concentrations in the liver well above the current action level for mercury in fish. The concentrations of aluminum, arsenic, silver, cadmium and lead were present in one-fourth to two-thirds of all samples and were at either comparable or below concentrations previously reported. Neither gender nor region had a significant effect on metal burdens. Future work should consider metal concentrations in juveniles and adults and toxicological studies need to be performed to begin to assess the toxicity of these metals.

DRAFTED for submission to ECOHEATLH: Health Assessment of Steller Sea Lions in Alaska, USA. Authors: Lieske, Camilla; Beckmen, Kimberlee; Burek, Kathy; Rea, Lorrie. Abstract #1: One hypothesis for the decline in the endangered western stock of Steller sea lions (Eumetopias jubatus) as compared to the threatened eastern stock is decreased pup survival rate. In conjunction with surveys for population dynamics, infectious disease prevalence and toxicologic exposure, methods for evaluating individual and population health are important evaluation tools. An objective, quantitative method of comparing individual and population health was developed as part of an epidemiological assessment of Steller sea lion health in Alaska, USA. Utilizing samples collected between 1998 and 2005, from sea lions aged one to 30 months, baseline ranges for hematology and blood chemistry parameters (hematocrit, white blood cell counts, total protein, albumin/globulin ratio, total bilirubin, BUN, creatinine, liver enzymes (ALT, AST, GGT), alkaline phosphatase, calcium, chloride, sodium, potassium, phosphorus, and glucose) were determined. These ranges were used to score different parameters, incorporating expected age differences and physiological associations (e.g. renal function score based on both BUN and creatinine). A total health score was calculated combining the blood parameters with physical examination findings. Overall, scores did not vary significantly (p>0.05) with age and sex, but scores did vary significantly by rookery, with a significant collection year/rookery interaction. No significant differences in pup or juvenile health was noted between the western and eastern stock.

ACCEPTED Journal of Wildlife Diseases (2008): **Dermoid Cysts in Caribou**. Authors: Wobeser, G., T. Bollinger, A. Neimanis, **K.B. Beckmen**. <u>Abstract #2</u>: Subcutaneous dermoid cysts were identified in eight wild caribou (*Rangifer tarandus*) from northern Canada and one wild caribou from Alaska. The dermoid cysts from Canadian caribou were found among 557 diagnostic specimens that had been detected by hunters and submitted by resource officers and biologists between 1 January 1966 and 15 May 2007. All of the cysts were located in the cervical region and five of nine were found in the throat area. Dermoid cysts were not diagnosed in any of 1108 white-tailed deer (*Odocoileus virginianus*), 293 mule deer (*Odocoileus hemionus*), 174 elk (*Cervus elaphus*) or 529 moose (*Alces alces*) examined during the same period at the Canadian laboratory.

Accepted for presentation at Ecohealth Forum in Anchorage Sep 2008. Serology and Genotyping of Morbillivirus for Arctic Fox and Polar Bears of Northern Alaska. Cassandra Knott, Kimberlee Beckmen, Todd O'Hara. Abstract #3. During the period of January through May of 2007, carcasses of twelve arctic fox (Alopex lagopus) were submitted from the north slope region to the Alaska Department of Fish and Game for necropsy evaluation. Foxes had been found dead or killed because of abnormal behavior or signs of illness and in vehicular collisions. -. Three of 12 animals tested positive for morbillivirus via RT-PCR and 2 of the 3 presented clinical signs and pathologic lesions consistent with canine distemper viral infection (details to be reported elsewhere). Two of six animals with brain samples suitable for testing were diagnosed with rabies; none tested to date were concurrently infected with morbillivirus. Phylogenetic analysis of a cloned 390 base pair fragment of the highly conserved phosphoprotein gene were performed for each of the three arctic fox cases whose sequences were determined to be identical. Alignment with sequences available from GenBank revealed that the arctic fox isolate differed from strains derived from two American dogs (Missouri, June through October of 2004) by only 1 nucleotide transition (G to A). The arctic fox sequence also displayed very high homology with five other sequences derived from Siberian seals (Phoca siberica) infected with CDV in Lake Baikal, Russia throughout 1988-1992 and also 1 sequence derived from an Alaskan dog during an outbreak which occurred among sled dogs in Kotzebue (Maes et al., 2003). The arctic fox isolate differed by only 2 nucleotides (both transitions and transversions) from each of these sequences. Interestingly, of the seven confirmed CDV cases examined during the 2004 Missouri outbreak, the 2 strains demonstrating high homology with the arctic fox strain examined in this study were determined by authors (Pardo et al., 2005) to be genetically distinct from viruses previously detected within the continental Unites States and most closely related to a Siberian seal isolate. The dogs originated from a breeder within the state and had no history of recent travel. Furthermore, when surviving arctic fox (N=11, approximately 3 months post epidemic) were sampled the following summer, the crossreactivity profile of antibodies detected via differential serum neutralization was not typical for that of terrestrial CDV. Ninety point one (10/11) of foxes tested positive for phocine distemper virus (PDV), 72.7% (8/11) for dolphin morbillivirus (DMV), 54.6% (6/11) for CDV, and 36.4% (4/11) tested positive for porpoise morbillivirus (PMV). These results are in contrast to that found for polar bears (sampled 2005 through 2007 in the southern Beaufort Sea, N=136) where prevalence rates were 24.3%, 4.4%, 50%, and 4.4% for PDV, DMV, CDV, and PMV, respectively. In polar bears, the greatest number of animals neutralized CDV and each animal positive for any of the other three viral antibodies demonstrated a higher titer to CDV. Viral nucleic acid has not been successfully isolated from polar bears or any other Ursid species to date, however serology alone suggests that the virus circulating among polar bears is distinct from the virus which infected compatric arctic foxes on the north slope of Alaska in 2007. Until viral nucleic acid can be derived and examined from other carnivores on the north slope of Alaska (eg. ice seals, polar and brown bears), the epidemiology of morbillivirus(es) circulating among these populations will remain unclear.

DRAFTED: **Fecal and oral aerobic bacteria of Alaska Steller sea lions** (*Eumetopias jubatus*). Sebastian E. Carrasco, Kathy Burek, Kimberlee Beckmen, Dr. Jonna Mazet. <u>Abstract #4</u>. Bacteriologic cultures from oral, rectal, and lesion samples collected from

free-ranging Steller sea lion (SSL, Eumetopias jubatus) pups and juveniles over 5 yr were examined retrospectively to determine the frequency of common and pathogenic aerobic bacteria isolated among eastern and western stocks of SSLs in Alaska. Associations between isolated aerobic bacteria and age, sex, body condition, location and sampling season were investigated. Salmonella spp. (n=49) were analyzed to determine spatial clustering and to identify serotypes (n=13) and antimicrobial susceptibility patterns (n=11). A total of 356 SSL pups (n = 272) and juveniles (n = 84) were sampled and 944 isolates were identified representing 13 different bacterial genera. Pasteurella spp. (43.8%), Streptococcus spp. (30.6%), and Mannheimia spp. (18.2%) were the most commonly isolated oral bacteria whereas Escherichia coli (74.1%), Salmonella spp. (12.3 %), and *Campylobacter* spp. (9.0 %) were the most frequently isolated bacteria from the rectum. Juveniles were more likely to test positive to Campylobacer spp. and E. coli eaeA gene. Pups from eastern stocks were more likely to test positive to *Campylobacter* and juveniles from eastern stocks were more likely to harbor the *E coli* eaeA gene. Salmonella was commonly associated with Pups from western stocks and samples collected during fall/winter seasons. A statistically significant cluster was detected at Perry Island haulout where 29 of the 49 rectal Salmonella isolates were noted. Five serotypes were isolated: Enteritidis, Infantis, Newport, Reading, and Stanley and some isolates were highly susceptible to ampicillin, ceftiofur, and enrofloxacin. These findings provide an unprecedented opportunity to identify rectal and oral aerobic microbial flora and to obtain baseline data about the antimicrobial susceptibility of Salmonella spp. Further molecular characterization of Salmonella isolates may provide valuable information on the epidemiology of this pathogen in SSLs.

SUBMITTED Journal of Wildlife Diseases: Neospora caninum and Toxoplasma gondii seroprevalence in wildlife of Alaska. Authors: Erica Stieve, Kimberlee Beckmen, Steve Kania, and Sharon Patton. Abstract #5: Caribou populations in some regions of Alaska have suffered declining numbers. Many of these herds are utilized by subsistence hunters and are managed by the state as a valuable resource. Prevalence of diseases that may impact herd health and recruitment from year to year are relevant to management decisions aimed to protect the long-term viability of these herds. Neospora caninum and Toxoplasma gondii are two apicomplexan parasites that can cause neurologic disease and abortions in their intermediate hosts and less frequently cause disease in their definitive hosts. The definitive hosts of N. caninum and T. gondii are canids and felids, respectively, and prevalence in the environment is dependent on maintenance of the life cycle of each host. Serum samples from caribou (*Rangifer tarandus*, N=453), wolf (*Canis* lupus, N=269), moose (Alces alces, N=201), black-tailed deer (Odocoileus hemionus, N=55), coyote (Canis latrans, N=12), and fox (Vulpes vulpes, N=9) collected in Alaska were screened and titered for N. caninum and T. gondii with an immunofluorescent antibody (IFAT) assay and a modified agglutination test (MAT), respectively. Seroprevalence of N. caninum and T. gondii were 16.7 % and 0.0 % in coyotes and 0.0% and 12.5 % in fox, but low sample size prevented further analysis. Seroprevalence of N. *caninum* was greater in caribou (11.5%) than in wolves (5.6%) or moose (0.5%). Seroprevalence of *T. gondii* was greater in wolves (13.6%) than in caribou (0.4%), moose (0%), or black-tailed deer (0%). Difference in moose and caribou may reflect a difference in the primary mode of transmission. Antibodies to N. caninum in young caribou compared to adult caribou suggest that vertical transmission may be an important

component of new infections in Alaskan caribou. The spatial distribution of seropositive individuals across Alaska reflects differences in frequency of definitive hosts and possible shifting of prey items from region to region.

ACCEPTED for publication in Virus Research: Genomic characterization of novel marine vesiviruses from Steller sea lions (Eumetopias jubatus) from Alaska. Authors: McClenahan, Shasta D.; Kathy A. Burek; Kimberlee B. Beckmen; Nick J. Knowles; John D. Neill, and Carlos H. Romero. Abstract #6: Marine vesiviruses were isolated in cell culture from oral and rectal swabs and vesicular fluid from Alaskan Steller sea lions (SSL; Eumetopias jubatus). Further characterization by RT-PCR, complete genomic sequencing, and phylogenetic analyses indicated that these viruses are most closely related to the marine vesiviruses, but are distinct viruses and represent two novel genotypes. The complete genome of these two SSL isolates was sequenced after cloning their viral cDNA. The genomes were found to be 8302 and 8305 nucleotides in length, organized in three open reading frames and contained 5' and 3' untranslated regions (UTR) of 19 and 180 nucleotides, respectively. The complete genomes of both SSL viruses were most closely related to each other and shared 83.0 % nucleotide identity. Using the very limited number of complete genomic vesivirus sequences available in the NCBI database, these novel SSL vesiviruses seem most closely related to vesicular exanthema of swine virus-A48 and least related to rabbit vesivirus and walrus calicivirus. Specific antiserum against some evolutionary closer marine vesiviruses did not neutralize these isolates supporting the novel nature of these SSL viruses.

SUBMITTED: Effect of Field Anesthesia and Cold Storage on Blood Cholinesterase Activity in Alaskan Caribou (*Rangifer tarandus granti*). Authors: Cristina M. Hansen, Petra A. Volmer, Kimberlee B. Beckmen. <u>Abstract #7:</u> Objective – to determine normal whole blood cholinesterase activity in caribou (*Rangifer* tarandus), and to determine the effects of injectable anesthetics (carfentanil and xylazine vs. xylazine) and freezing on blood cholinesterase activity. Summary: Sample Population – 129 wild Alaskan caribou and 9 captive caribou housed in Alaska.

Procedures - 158 whole blood samples from 6 herds of wild caribou and a small group of captive animals were utilized. Cholinesterase was analyzed using a modification of the Ellman colorimetric method. Enzyme activities were statistically analyzed using sedative, storage, sex, and age as variables.

Results - Whole blood cholinesterase activities ranged from $1.05 - 3.68 \mu mol/ml/min$. There was a statistically significant increase in cholinesterase activity in animals that were sedated with a combination of carfentanil and xylazine compared to those that were not sedated, or who were sedated with xylazine alone. Frozen samples had a significantly lower cholinesterase activity than fresh samples. Calves had a significantly lower activity than yearlings and adults. There was no statistical difference between the activity of males and females.

Conclusions – The use of field anesthetics for the collection of blood, and the freezing of blood for storage, effect blood cholinesterase activity. Although changes in cholinesterase activities in this study have statistical significance, the clinical significance is considered to be minor.

DRAFTED AND READY FOR SUBMISSION to Marine Pollution Bulletin in FY09: Organochlorine contaminant concentrations in multiple tissue matrices of live Steller sea lions (*Eumetopias jubatus*) in Alaska. Authors: Beckmen, K.B., K.A. Burek, K. W. Pitcher, G. M. Ylitalo, and B.S. Fadely. Abstract #9: 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 these animals. The relationships of various OC contaminants in multiple matrices from individuals were examined to determine the appropriate matrix for exposure monitoring in live animals and to minimize invasive sampling techniques. Concentrations of certain OC contaminants in blubber, milk and blood were highly correlated within individuals; however fecal concentrations were only correlated with those measured in blood. These findings indicate that a whole blood sample may be the best alternative as a less-invasive indicator of relative contaminant exposure in lieu of surgical blubber biopsy. Feces may be used as a non-invasive monitoring tool of relative OC exposure without direct handling of animals for sample collection.

DRAFTED AND READY FOR SUBMISSION To Emerging Infectious Diseases: Infection and mortality in wild and domestic birds due to Escherichia albertii: description of avian strains of an emerging pathogen. Authors: J. Lindsay Oaks, Thomas E. Besser, Kimberlee B. Beckmen, Kathy A. Burek, Gary H. Haldorson, Dan S. Bradway, Fred R. Rurangirwa, Margaret A. Davis, Greg Dobbin, Pierre-Yves Daoust, and Thomas S. Whittam. Abstract #10: A mortality event affecting common redpolls (Carduelis flammea) in Alaska led to the identification of Escherichia albertii as the probable cause. Subsequent investigation associated E. albertii with enteritis in other birds, including a falcon, a chicken, and detection in clinically normal finches. In addition, isolates from finch mortality events in Scotland previously identified as Escherichia coli O86:K61 were shown to be E. albertii. E. albertii is a recently described member of the *Enterobacteriaceae* associated with diarrheal illness in humans, but has not been previously associated with disease or infection in animals. Similar to the human isolates, the avian E. albertii isolates contained genes for eae (intimin) and cdt (cytolethal distending toxin), but lacked the genes for stx (Shiga toxins). Comparison of eae and cdt sequences, multilocus sequence typing, and pulsed field gel electrophoresis showed that the avian E. albertii strains are heterogeneous and distinct from human E. albertii isolates.

I attended several major international meetings, conferences, or symposia to present research findings and received continuing education credits required to retain veterinary licensure in Alaska. In chronological order these meeting included: 2007. 56nd Annual Wildlife Disease Association Conference and CWD Symposium Presenter and continuing education recipient, Aug 11-18, 2007, Estes Park, CO [Abstracts #8, #10, #11]

2007. XVI Biennial Conference of Marine Mammals, presenter, and attendee of preconference workshop on Conservation Medicine, Nov 27-Dec 5 2007, Cape Town, South Africa [Abstracts #12 ] 2008. Canadian National Wildlife Disease Surveillance Workshop. Feb 20-22, Calgary Alberta, Canada

Abstracts for presentations/posters and co-authored presentations/posters are below. I kept abreast of current research in wildlife disease through the literature.

Poster Presentation WDA 2007. Investigation of trace mineral deficiencies in an Alaskan Dall's sheep population. Kimberlee Beckmen, Jim Herriges, Jim Lawler, Mark Bertram. <u>Abstract #9</u>. A

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Abstract #8: A study of trace mineral status in Dall Sheep (Ovis dalli dalli) from the White Mountains of Alaska was undertaken to discover if the occurrence of 'stump headed" horn abnormalities was related to a deficiencies of minerals. Two spinal fracture/capture myopathy deaths occurred in vitamin E and selenium deficient adult females occurred and 3-week post-capture mortality rates were significantly higher than in other regions of Alaska (11.2% vs. 2.7%). The two spinal fractured sheep had osteoporosis and osteopenia, however serum and liver calcium levels were not correlated with horn abnormalities or fractures in captured sheep. Serum, blood and hair samples from 48 sheep were analyzed for trace minerals. White Mountains (WM) sheep samples had marginal levels of selenium and were significantly lower than sheep from Lake Clark National Park (LK). Copper concentrations in WM sheep hair samples were marginal to deficient. However, in serum, LC sheep had significantly lower mean copper concentrations than WM. Compared to domestic sheep, 80% of LC and 31% of WM sheep were copper deficient. Mean serum zinc from WM sheep was significantly lower than LK sheep and 81% vs. 57%, respectively, were marginal or deficient compared to domestic sheep. Hair was also marginal to deficient in zinc compared to domestic sheep. The addition of vitamin E/selenium injections at capture, intranasal xylazine injections and conducting net-gun capture operations mainly in the fall, significantly reduced capture mortality rate and decreased the incidence of capture myopathy. The proximate cause of horn abnormalities remains to be elucidated.

Poster Presentation WDA 2007. Effects of Capture Method on Blood Parameters in Caribou: Evaluation of Physical and Chemical Immobilization Techniques. Authors: Kimberlee Beckmen, Jennifer Schmidt, Mark Keech, and Bruce Dale. <u>Abstract #10.</u> The adverse effects of capture stress and potential for mortalities are a concern when capturing cervids. In this study we evaluated the effects of different capture protocols, 2 physical and 2 chemical, on various blood parameters related to capture stress. The physical capture methods were net-gunning from a helicopter (n=16) and hand-capture with manual restraint from boats during a river crossing (n=20). Chemical combinations evaluated were carfentanil/xylazine (n=54) and ketamine/medetomidine (n=9). Drugs were administered via helicopter darting. Blood parameters used to quantify stress and

risk of capture myopathy included lactate and creatinine phosphate (CPK). Use of a handheld lactate monitor was validated for field use in caribou with a significant correlation to laboratory serum lactate (p>0.0001). Mean serum CPK concentrations were significantly higher in hand-captured caribou than in chemically-immobilized caribou. Caribou darted with a ketamine/medetomidine had the lowest mean CPK and lactate concentrations but only the latter was significantly different from other capture methods. Increased lactate was significantly correlated with increasing chase time (r=0.46, p<0.0001). Mean lactate levels in physically captured caribou were nearly three times that of chemically immobilized caribou. These data indicate that physical capture methods appear to be more 'stressful' for caribou than helicopter darting and thus may pose a higher risk for capture myopathy. Furthermore, the drug combination of ketamine/medetomidine appears to at least as safe as carfentanil/xylazine and eliminates the need for the controlled narcotic. Body condition, season, and reproductive state also need to be considering how and when to capture caribou.

Oral Presentation WDA 2007: Managing the adverse impacts of the biting dog louse on wolves in Alaska. Authors: Kimberlee Beckmen, Craig Gardner, Mark May. Abstract #11: The biting dog louse (Trichodectes canis) was first identified in Alaska on a coyote (Canis latrans) and wolves (C. lupus) on the Kenai Peninsula during the winter of 1981-82. Wildlife managers attempted to eliminate it by administrating ivermectin injections during live-capture and with ivermectin-treated baits. This effort was unsuccessful because of the difficulty in treating all exposed individuals. In 1998, trappers reported wolves and coyotes with lice in south-central. Treatment attempts were similarly unsuccessful. When lice were detected in wolves north of the Alaska Range in March 2004, a cost-effective means of management rather than eradication of lice infestation was sought. In spring 2005 all members of an infected pack were radiocollared and treated. After emergence from the den, pups were treated at two week intervals with ivermectin-impregnated baits dropped from a Supercub. In December, pups were live-captured, ear-tagged assessed for lice, and released. Pelts from marked wolves obtained from trappers were subjected to hide digestion to detect occult infestations. The pack was determined to be louse-free. During 2005-06, 1-2 wolves in each of 11 packs were examined and radio-collared. Ivermectin-baits were distributed at the dens and rendezvous sites of 5 infected packs during May-August. During the winter, 1-3 wolves were examined from each of 8 radio-collared packs. None of the wolves from treated packs had lice or hair loss suggestive of lice. Preliminary results suggest that distribution of ivermectin-impregnated baits is efficacious in production normal pelt quality wolves during the trapping season.

Poster Presentation MMC 2007. **Organochlorine, pesticides and polybrominated diphenyl ether contaminant concentrations in multiple tissue matrices of live Steller sea lions** (*Eumetopias jubatus*) in Alaska. Authors: Beckmen, Kimberlee B.; Burek, Kathleen A; Pitcher, Kenneth W.; Ylitalo, Gina M.; Fadely, Brian. <u>Abstract #12.</u> Blood, blubber, milk, and feces were collected from 53 free-ranging and 3 captive Steller sea lions (*Eumetopias jubatus*) in Alaska from 1998-2003 to assess exposure to selected organochlorine (OC) contaminants (e.g., dioxin-like PCBs, DDTs). Organochlorine contaminant relationships among multiple matrices of individuals were used to determine the appropriate tissue for exposure monitoring in live animals and thus minimize invasive

sampling techniques. Concentrations of certain OC contaminants in blubber, milk and blood were highly correlated within individuals; however fecal concentrations were only correlated with those measured in blood. Thus a blood sample may be the best alternative as a less-invasive indicator of relative contaminant exposure in lieu of surgical blubber biopsy while feces may be used as a non-invasive monitoring tool of relative OC exposure without direct handling of animals. Regional OC contaminant exposure was compared in blubber samples of pups through sub-adults of the stable eastern stock in Southeast Alaska (n=48) as compared to the endangered western stock of the Gulf of Alaska (n=55) and Aleutian Islands (n=43). Pesticides and polybrominated diphenyl ethers were detected in 25 and 15 animals respectively, including 4 individuals that were sampled at 5 month intervals. Transplacental transfer of OCs was extremely low. Concentrations of OCs peaked in pups sampled between 2 - 6 weeks of age, declined by midway through the suckling period, and increased again through the first year of the presumed dependent suckling period though the weaning period. These data suggests that exposure to OCs is at a level of concern especially in young pups in portions of the range of the endangered western stock of Steller sea lions.

Poster Presentation MMC 2007. Health Assessment of Steller Sea Lions in Alaska, USA. Authors: Lieske, Camilla; Beckmen, Kimberlee; Burek, Kathy. Abstract #13. One hypothesis for the decline in the endangered western stock of Steller sea lions (Eumetopias jubatus) as compared to the threatened eastern stock is decreased pup survival rate. In conjunction with surveys for population dynamics, infectious disease prevalence and toxicologic exposure, methods for evaluating individual and population health are important evaluation tools. An objective, quantitative method of comparing individual and population health was developed as part of an epidemiological assessment of Steller sea lion health in Alaska, USA. Utilizing samples collected between 1998 and 2005, from sea lions aged one to 30 months, "normal" ranges for hematology and blood chemistry parameters (hematocrit, white blood cell counts, total protein, albumin/globulin ratio, total bilirubin, BUN, creatinine, liver enzymes (ALT, AST, GGT), alkaline phosphatase, calcium, chloride, sodium, potassium, phosphorus, CO2 and glucose) were determined. These ranges were used to score different parameters, incorporating expected age differences and physiological associations (e.g. renal function score based on both BUN and creatinine). A total health score was calculated combining the blood parameters with physical examination findings. Overall, scores did not vary significantly (p>0.05) with age and sex, but scores did vary significantly by rookery, with a significant collection year/rookery interaction. No significant difference in pup and juvenile health was noted between the western and eastern stock.

Presentations by Collaborators:

National Institutes of Health Graduate Student Research Festival, October 10-12, 2007. Poster Presentation: **Detection and Molecular Characterization of Marine Caliciviruses.**

Shasta D. McClenahan¹, Kathy A. Burek², Kimberlee B. Beckmen³, John D. Neill⁴, Alvin W. Smith⁵, and Carlos H. Romero¹

¹ University of Florida; ² Alaska Veterinary Pathology Services; ³ Alaska Department of Fish and Game; ⁴ USDA-National Animal Disease Center, ⁵ Oregon State University

<u>Abstract #14</u>: The <u>Caliciviridae</u> family is a diverse group of viruses that infect a wide variety of hosts including humans and many animal species. Viruses in the <u>Vesivirus</u> genus, unlike the human caliciviruses, can be grown in cell culture and are therefore important surrogates for studying calicivirus biology. Vesiviruses are also economically important due to the vesicular disease produced in livestock, and zoonotic potential. The purpose of this research project was to molecularly characterize novel isolates of marine vesiviruses and develop new diagnostic techniques for the detection of these viruses.

Two novel marine vesiviruses were isolated from sea lions in Alaska. Electron microscopy, RT-PCR, and sequencing confirmed the isolates to be members of the Vesivirus genus. The full genomes were sequenced for both isolates after PCR amplification and cloning using primer sets designed targeting conserved regions of vesivirus sequences from the GenBank database. Phylogenetic trees were constructed using Genetic software to determine genetic relatedness with other members of the Caliciviridae.

A diagnostic, real-time RT-PCR assay was developed for the detection of marine vesiviruses, targeting a conserved region within the capsid gene. This assay was found to be specific for only the marine vesiviruses, as it amplified ten marine vesiviruses, but failed to amplify feline calicivirus, a closely related vesivirus. The assay described here can be used as a diagnostic tool to rapidly identify and differentiate marine vesiviruses from other viruses that cause vesicular diseases in livestock, and zoonotic disease in humans.

Third International Calicivirus Conference, November 10-13, 2007, Cancun, Mexico. Poster Presentation: Marine Vesiviruses: Detection and Characterization of Novel Genotypes

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<u>Abstract #15:</u> The *Vesiviruses* are a diverse group of animal viruses that are economically important due to their impacts of vesicular disease in livestock, and their zoonotic potential. More than 40 serotypes of vesiviruses from the marine environment have been described, including San Miguel sea lion virus (SMSV) and vesicular exanthema of swine virus (VESV). Historically these viruses have caused vesicular disease and reproductive failures in both aquatic and terrestrial hosts. The goal for this project was to isolate and characterize vesiviruses from marine mammals to determine if novel genotypes are emerging, and their association, if any, with disease. Population declines of Steller sea lions (SSL) have lead to the search for microbial agents that may be responsible, including caliciviruses. Samples were collected for virus isolation including oral and rectal swabs, vesicular fluids, tissues, feces, and serum from SSL in Alaska from 2001-2005. Nine vesiviruses were isolated in cell culture, visualized by electron microscopy, and verified to be members of the Vesivirus genus following RT-PCR and direct sequencing of a 768-bp RT-PCR product from the capsid gene.

Sequence analysis revealed two novel genotypes, and the complete genomic sequences from both viruses were obtained for characterization.

Serological surveys were conducted by virus neutralization in order to determine the spread of these novel genotypes within the SSL populations. Sera collected from SSL indicate that new genotypes are temporally emerging in populations and replace older viruses. This is most likely due to the increased fitness of the new virus, and lack of neutralizing antibodies to the new genotype.

These new vesiviruses are being used to develop improved diagnostic assays for the detection of marine vesiviruses. These assays include a real-time RT-PCR assay for the rapid and sensitive detection of any of the marine vesiviruses, and serological assays using virus-like particles (VLP) expressed from the capsid gene.

American Society for Virology 26th Annual Meeting, July 14-18, 2007, Corvallis, Oregon. Oral Presentation : DEVELOPMENT OF A REAL-TIME RT-PCR ASSAY FOR THE DETECTION OF MARINE CALICIVIRUSES

McClenahan, Shasta D.¹; Neill, John D.²; Burek, Kathy A.³; Beckmen, Kimberlee B.⁴; and Romero, Carlos H.¹

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Abstract #16: More than forty different marine calicivirus serotypes (family Caliciviridae, genus Vesivirus) have been identified from marine and terrestrial hosts since their initial isolation. Marine vesiviruses have previously infected swine along the Western coast of the United States and induced maladies clinically indistinguishable from foot-and-mouth disease and other vesicular swine diseases. Current methods for identification of marine vesiviruses include RT-PCR and serological assays. These assays may fail to identify all positive clinical samples, due to the high mutation rate of these RNA viruses and the resulting large number of serotypes and genotypes. We have developed a rapid and differential diagnostic real-time RT-PCR assay to identify marine vesiviruses. Primers were designed to amplify a conserved 176 nucleotide fragment of the capsid gene, based on multiple alignments of Vesivirus sequences obtained from the GeneBank database and two novel marine vesiviruses recently identified in our laboratory. An oligonucleotide probe, sixteen bases in length, was designed based on sequences within the amplicon and labeled at one end with a reporter fluorescent dye and at the other end with a fluorescence quencher. The probe includes locked nucleic acid (LNA) nucleotides to increase the melting temperature and stability of the probe in the assay. This assay accurately identified ten different marine vesiviruses grown in cell culture. These viruses included: San Miguel sea lion virus (SMSV) type 1, SMSV-2, SMSV-4, SMSV-5, SMSV-6, SMSV-13, SMSV-14, bovine calicivirus (Bos-1), and two novel Steller sea lion vesiviruses. The assay was shown to be specific for only marine

vesiviruses, as it did not cross-react with three isolates of feline calicivirus (FCV), a different species within the Vesivirus genus. The assay described here can be used as a diagnostic tool to rapidly identify and differentiate marine vesiviruses from other viruses that cause vesicular diseases.

Federal funds were used to pay salaries, supplies and services on this task.

JOB/ACTIVITY 7: Perform the duties of Attending Veterinarian.

I trained, assisted and conducted wildlife capture operations including Western Arctic Caribou Sept 10-15, 2007; North Slope muskoxen 103-4, 2007; Steller sea lions in Prince William Sound Nov 11-19, 2007 and 42-17, 2008, Seward Peninsula muskoxen March 23-30, 2008. I oversaw the anesthesia and Taser research trials at the Moose Research Center to ensure compliance with humane treatment. I purchased, prescribed and dispensed animal capture drugs to DWC personal. I attended Avian Influenza Response Training in Anchorage and I conducted Avian Influenza Preparedness training for Biologists. I gave advice and information to the public and DWC employees related to wildlife health and zoonotic diseases via personal contact in the office, on the phone and through the media. I served as chair or the DWC Animal Care and Use Committee (ACUC) as well as the attending veterinarian for the committee to assure Division compliance with the Animal Welfare Act. I conducted a veterinary review of 20 Assurances of Animal Care Protocols submitted to the DWC ACUC prior to committee review. I conducted IACUC Facility inspections of the Moose Research Center, the Moose/caribou research herd held at the UAF Ag facility in Palmer, and the Wood Bison enclosures at the Alaska Wildlife Conservation Center near Portage. I attended and taught at the IACUC Advanced Workshop in Anchorage Sept 4-5, 2007. I continued to maintain a strong, mutually beneficial relationship as a liaison between ADF&G and the Department of Health and Human Services/Division of Epidemiology and the Division of Environmental Conservation/Office of the State Veterinarian.

Federal funds were used to pay salaries, supplies and services on this task.

III. ADDITIONAL FEDERAL AID-FUNDED WORK NOT DESCRIBED ABOVE THAT WAS ACCOMPLISHED ON THIS PROJECT DURING THE LAST SEGMENT PERIOD, IF NOT REPORTED PREVIOUSLY

Not applicable

IV. PUBLICATIONS

[see Appendix C]: Canadian Journal of Zoology. (2007) 85:1143-1156. Serendipitous discovery of a novel protostrongylid (Nematoda: Metastrongyloidea) in caribou (*Rangifer tarandus*), muskoxen (*Ovibos moschatus*) and moose (*Alces americanus*) from high latitudes of North America based on DNA sequence comparisons. Authors: Kutz, SJ., I. Asmundsson, E. P. Hoberg, G. D. Appleyard, E. J. Jenkins, K. Beckmen, M.. Branigan, L. Butler, N. B. Chilton, D. Cooley, B. Elkin, F. Huby-Chilton, D. Johnson, A. Kuchboev, J. Nagy, M. Oakley, R. Popko, A. Scheer, M. Simard, A. Veitch.

[see Appendix D] Acta Parasitologica, 2007, 52(4), 299–304. *Sarcocystis arctosi* sp. nov. (Apicomplexa, Sarcocystidae) from the brown bear (*Ursus arctos*), and its genetic

similarity to schizonts of *Sarcocystis canis*-like parasite associated with fatal hepatitis in polar bears (*Ursus maritimus*). Authors: J.P. Dubey, Benjamin M. Rosenthal1, Natarajan Sundar1, G.V. Velmurugan1 and Kimberlee B. Beckmen.

[see Appendix E]: Marine Pollution Bulletin (2008) 56:1416-1421. **Metal Tissue Levels in Steller Sea Lion** (*Eumetopias jubatus*) **Pups.** Authors: Amie L. Holmes, Sandra S. Wise, Caroline E. C. Goertz, J. Lawrence Dunn, Frances M. D. Gulland, Tom Gelatt, **Kimberlee B. Beckmen,** Kathy Burek, Shannon Atkinson, Mary Bozza, Robert Taylor, Tongzhang Zheng, Yawei Zhang, AbouEl-Makarim Aboueissa, John Pierce Wise, Sr.

V. RESEARCH EVALUATION AND RECOMMENDATIONS Briefly evaluate your approach to this study. Would you make changes? If so, list recommended changes to this study or related studies; suggest new research techniques and possible new areas of research related to this project that could enhance game management. Note: this is not a Federal Aid reporting requirement.(optional)

Disease surveillance and veterinary activities have steadily increased in scope and intensity over the course of this performance period. This is an important trend that should be continued. However, for this to occur, enhanced staffing levels and funding must coincide. Federal funding of CWD surveillance is decreasing and it is no longer sufficient to maintain adequate surveillance of free-ranging cervids in Alaska. Likewise, funding for West Nile Virus surveillance is no longer available for Alaska. These deficiencies will need to be mitigated by other funding sources including Federal Aid. Additional field and captive studies testing the effects of diseases and parasites on wildlife health are needed to understand the role of these factors on populations so they can be manipulated as needed for management purposes.

VI. APPENDIX

Attached.

PREPARED BY:

Kimiberlee S. Beckmen

<u>Kimberlee Beckmen</u> Wildlife Veterinarian

SUBMITTED BY:

<u>Scott Brainerd</u> Research Coordinator APPROVED BY:

Clayton Hawkes Federal Aid Coordinator Division of Wildlife Conservation

Douglas N. Larsen, Director Division of Wildlife Conservation

APPROVAL DATE: _____