# 68<sup>th</sup> ANNUAL WILDIFE DISEASE DISEASE ASSOCIATION INTERNATIONAL CONFERENCE

August 4-9, 2019 Tahoe City, California, USA

## **Fostering Resiliency in a Time of Change**

# Wildlife Disease Association

Fostering resiliency in a time of change

68th Annual International Conference Tahoe City, California, USA August 4-9, 2019

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Pieter Johnson

University of Colorado

# Abstract

Why disease biology needs community ecology: linking biodiversity changes with parasite transmission.

# WILDLIFE HEALTH PROFESSIONALS SHIFTING THE PARADIGM FOR ADDRESSING EMERGING INFECTIOUS DISEASES: RESPECT OR RESPONSIBILITY?

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## Abstract

Wildlife health professionals shifting the paradigm for addressing emerging infectious diseases: Respect or Responsibility?

## ARE WE READY FOR AFRICAN SWINE FEVER?

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## Abstract

African swine fever virus (ASFV) has emerged for the second time in Europe. Native Eurasian wild boar (*Sus scrofa*) maintain ASFV circulation even in the absence of domestic pigs. Factors explaining this are those driving wild boar overabundance. We address ASF preparedness in non-infected countries taking advantage of lessons learned from the Czech outbreak. Actions needed in this context include improved wild boar monitoring, measures for wild boar population control, and outbreak preparedness.

To improve wild boar population monitoring, www.enetwild.com works on three aspects: (1) collecting data on distribution and abundance, particularly hunting harvest; (2) engaging hunters and authorities in recording hunting data at event level; and (3) developing camera-trap grid protocols to assess wild boar abundance independently of hunting.

Regarding wild boar control, meetings and awareness campaigns help in engaging hunters in aspects of biosafety and wild boar control, and articles in the media inform the public about the negative consequences of overabundance. However, it is unclear which wild boar control strategies can sustainably reduce the current densities of this native species, and whether this is feasible in a context of increasing habitat carrying capacity and changing society.

Outbreak preparedness includes farm biosafety, improved disease surveillance protocols and field exercises on carcass detection, sampling and destruction. The Czech experience shows that it is possible to eradicate ASFV in isolated outbreaks. The key action is the systematic search and rapid removal of carcasses. Hunting should be carried out in the vicinity of the infection center and start immediately after the zones have been defined. Involving recreational hunters in dealing with ASF requires communication, transparency and team-building. The rules of hunting in an infected area must be simple, clear and easily verifiable.

ASF control requires coordinated wildlife and disease management strategies. This is a contribution to grant CGL2017-89866 and Fundación Biodiversidad.

# SIMPLE SOLUTIONS, COMPLEX PATH: UTILIZING PARTICIPATORY EPIDEMIOLOGY TO HALT THE PPR VIRUS EPIDEMIC IN LIVESTOCK AND ENDANGERED WILDLIFE ON THE MONGOLIAN STEPPE

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# Abstract

Peste des petits ruminants (PPR) is a highly contagious viral disease of wildlife and domestic small ruminants that is spreading rapidly around the world. The global impacts of PPR are estimated to be up to US\$2.1 billion annually (FAO/OIE, 2016) and the disease has been targeted for global eradication by 2030. Losses of livestock due to PPR are associated with damage to livelihoods, food security, and nutrition for millions of small-scale farmers and pastoralists. PPR's emergence in Mongolia in 2016, highlighted the additional impacts of PPR on biodiversity conservation. The spillover of the virus from livestock to wildlife triggered a die-off of critically endangered Mongolian saiga antelope (Saiga tatarica mongolica) that reduced the population by 66% in under two months, significantly setting back years of conservation investment. The successful control and eradication of PPR is linked to multiple aspects of human well being and tangible conservation outcomes. The SNAPP Steppe Health interdisciplinary team is working across multiple jurisdictions, with multiple actors, to synthesize information and define the disease situation in the ungulate community to identify practical solutions to the major challenges facing the health authorities, the livestock sector, and conservationists working to halt this epidemic occurring at the livestock/wildlife interface. Participatory epidemiology is piloted in Mongolia to gather critical information and incorporate local knowledge into more traditional approaches to data collection, data analysis, and disease risk assessment. The local knowledge gathered from a diverse set of actors and key informants contributes to our understanding of disease dynamics at the wildlife/livestock interface. Initial results indicate that the incorporation of local knowledge improves the science and empowers people to take action to implement their own solutions to the challenges of managing PPR, thereby improving livelihoods and preserving Mongolia's biodiversity.

## DETECTION OF MARBURG VIRUS IN ROUSETTE BATS IN SIERRA LEONE

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# Abstract

Marburg virus (MARV), first discovered in 1967, causes sporadic outbreaks of Marburg virus disease (MVD) in Africa. This filovirus, until recently, has primarily been associated with populations of Egyptian rousette bats (Rousettus aegyptiacus), the natural reservoir host, in East and South Africa and also Gabon. Moreover, all but one of the 12 known MVD outbreaks have occurred in, or originated from, Uganda, eastern Democratic Republic of the Congo (DRC) or northern Zimbabwe. The one exception was the MVD outbreak that occurred in Uige, Angola in 2005. This was the largest MVD outbreak on record with 252 confirmed cases and 227 deaths, which is the highest case-fatality ratio (CFR: 90%) recorded for any large filovirus outbreak, including the West African Ebola outbreak (CFR: 41%). Direct links to an environmental spillover event in Angola were never established. Recently, simultaneous filovirus survey efforts by the Centers for Disease Control and Prevention in collaboration with Njala University and the University of California, Davis USAID-PREDICT project in collaboration with University of Makeni have identified the presence of MARV circulating in Egyptian rousette populations (11/692; 1.6%) in Sierra Leone by real-time RT-PCR and consensus RT-PCR. Sequences obtained from bat samples align closely with the Angola strain of MARV as well as to another strain that has been detected in bats from Gabon and the Democratic Republic of the Congo. These data are the first definitive evidence of MARV circulation in West Africa and the first report of the MARV Angolan strain in bats. Importantly, this discovery occurred in the absence of, and prior to, any known MVD outbreak in Sierra Leone and highlights the value of a broad surveillance approach. This finding also raises the awareness to include Marburg virus on the list of differentials for diagnostic testing of hemorrhagic disease cases in West Africa.

# DISCOVERY OF AN EBOLAVIRUS IN BATS ADDS FURTHER SUPPORT FOR BATS AS HOSTS OF EBOLAVIRUSES

Tracey Goldstein<sup>1,4</sup>, Simon Anthony<sup>2</sup>, Aiah Gbakima<sup>3</sup>, Brian Bird<sup>1,4</sup>, James Bangura<sup>1</sup>, Alexandre Tremeau-Bravard<sup>1</sup>, Manjunatha Belaganahalli<sup>1,4</sup>, Heather Wells<sup>2</sup>, Jasjeet Dhanota<sup>1,4</sup>, Eliza Liang<sup>2</sup>, Michael Grodus<sup>2</sup>, Rohit Jangra<sup>5</sup>, Veronica DeJesus<sup>5</sup>, Gorka Lasso<sup>2</sup>, Brett Smith<sup>1,4</sup>, Amara Jambai<sup>6</sup>, Brima Kamara<sup>6</sup>, Sorie Kamara<sup>7</sup>, William Bangura<sup>7</sup>, Corina Monagin<sup>1,4</sup>, Sagi Shapira<sup>2</sup>, Christine K. Johnson<sup>1,4</sup>, Karen Saylors<sup>8</sup>, Eddy Rubin<sup>8</sup>, Kartik Chandran<sup>5</sup>, Ian Lipkin<sup>2</sup>, Jonna A.K. Mazet<sup>1</sup>

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#### Abstract

Bats are thought to be hosts for ebolaviruses, but despite more than 40 years of research and continued outbreaks, the reservoirs of ebolaviruses remain unknown and outbreaks continue to occur. We initiated a survey in Sierra Leone, Guinea and Liberia to identify hosts of Ebola virus (EBOV; species Zaire ebolavirus) and to identify any additional filoviruses that might be circulating in wildlife. Oral, rectal, and/or blood samples were collected from live captured bats, rodents, dogs, and cats, and consensus PCR targeting all filoviruses was used to screen for EBOV and any novel filoviruses. High-throughput sequencing and genome walking were used to recover complete viral genomes and in vitro experiments were conducted to evaluate viral ability to mediate entry into human cells. Findings included the detection of a novel ebolavirus in free-tailed bats (Chaerephon pumilus and *Mops condylurus*) living in close proximity to humans. Phylogenetic analyses showed that the virus is sufficiently distinct to represent the prototypic strain of a new species within the Ebolavirus genus; and has been named Bombali virus (BOMV; species *Bombali ebolavirus*). Using a vesicular stomatitis virus expression (rVSV) system encoding the BOMV GP gene, we showed that the rVSV- BOMV GP was infectious in human osteosarcoma cells demonstrating that the BOMV GP is fully competent to mediate viral entry suggesting the virus has zoonotic potential. The bats were also found roosting inside houses, indicating the potential for human transmission. This effort has led to recent major discoveries that have improved our understanding of the ecology and potential health risks from EBOV and related viruses. Findings of the first full genomes of ebolaviruses in bats provide strong evidence that bats are a natural reservoir of these viruses.

### SPILLOVER: A NEW TOOL FOR VIRAL RISK RANKING INCORPORATING WILDLIFE DATA

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#### Abstract

It is estimated that there are up to 827,000 undiscovered viruses in animals. Of this large pool, which viruses pose the greatest threat to humans? Conventional approaches to understanding the risk of viral spillovers from wildlife, such as relying on the genetic relatedness of viruses, have proven insufficient. We developed an interactive web tool called SpillOver to assess whether a wildlife virus is more or less likely to spillover and cause a new epidemic in humans. This app uses a combination of scientific evidence and expert opinion to produce a risk report for hundreds of wildlife viruses. The report details the relative risk of host, viral and environmental factors that may contribute to a given virus' overall spillover risk score. This approach allows comparison between viruses found in the USAID PREDICT wildlife surveillance project and known zoonotic viruses and identifies those with the highest potential for zoonotic transmission. The web application is intended for use by policy professionals, scientists and the general public to compare and explore the relative public health risk for wildlife viruses. This is the first-time a crowd-sourcing website has been developed in which all can collaboratively participate in the collation of data and viral risk ranking. As viral discovery efforts reduce the unknown burden of what is out there, and our understanding of viral outbreaks increases, SpillOver provides an adaptive platform to improve our perceived risk, with the ultimate aim of forecasting and preventing future disease outbreaks.

# PHOCINE DISTEMPER OUTBREAK AND THE 2018-2019 NORTHEAST UNITED STATES PINNIPED UNUSUAL MORTALITY EVENT

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#### Abstract

An Unusual Mortality Event (UME) was declared by the National Marine Fisheries Service on August 30, 2018 due to increased numbers of harbor seal (Phoca vitulina) and gray seal (Halichoerus grypus) strandings along the U.S. coasts of Maine, New Hampshire, and Massachusetts during July and August. Strandings have remained elevated in these three states and expanded south to Virginia with positive cases on-going. Recently, harp (Pagophilus groenlandicus) and hooded (Cystophora cristata) seals have begun stranding as they migrate from Canada into U.S. waters and have been included in the investigation. From July 1, 2018 to February 20, 2019, >1,740 harbor and gray seals stranded with 95% of the seals stranding in Maine, New Hampshire and Massachusetts. Live seals had evidence of respiratory and/or neurological disease, with pneumonia present on gross necropsy examination. Histologically, seals had bronchointerstial and/or suppurative pneumonia, meningoencephalitis or encephalitis, and/or lymphoid depletion consistent with morbillivirus infection. Tissue or swab samples from 65% (22 of 34) of seals tested to date via polymerase chain reaction (PCR) were positive for phocine distemper virus (PDV). Additionally, ~86% (6 of 7) of cases tested by immunohistochemistry (IHC) were positive for PDV in lung, lymph node or brain tissue. Serum was tested for PDV neutralizing antibody titers, with positive titers ranging from 64 to > 512. To date positive PCR, IHC or antibody titers for PDV were only detected in harbor and gray seals. Virus isolation and genome sequencing of the virus from this outbreak determined the sequences to be most similar to the 1988 and 2006 PDV isolates. Naïve live seals in rehabilitation are being vaccinated with the PUREVAX<sup>®</sup> ferret distemper vaccine prior to release to ensure protection and improve survival after release. Research is ongoing to understand the impacts of this large scale outbreak on seal population(s).

#### HARMFUL ALGAL BLOOMS IN NORTHERN WATERS: AN EMERGING ISSUE FOR ALASKAN SEABIRDS?

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## Abstract

Harmful algal blooms (HABs) produce biotoxins that can injure or kill shellfish, fish, wildlife, and humans. They occur naturally but have been increasing in frequency and severity worldwide due to recent climatic changes, including warming ocean temperatures. HABs pose a growing threat to northern marine ecosystems and associated wildlife and human inhabitants. During 2015–2016, an unprecedented die-off of Common Murres (Uria algae) in Alaska was observed in association with anomalously warm ocean waters in the NE Pacific. Subsequent die-offs of seabirds, including Northern Fulmars (Fulmarus glacialis), kittiwakes, puffins, and shearwaters, occurred in several locations throughout Alaskan waters between 2016 and 2018. Tissues of seabirds, both dead and apparently healthy, and forage taxa were tested for saxitoxin (STX) and domoic acid (DA). STX was common in both die-off and "healthy" birds across different seabird species, locations, seasons, and years. The occurrence and concentration of STX differed by species, and concentrations varied by tissue type. In contrast, DA was found rarely in seabirds and only in trace concentrations. Detectable levels of STX and DA were also observed frequently in forage taxa. Although toxic thresholds have not been established for birds, our research has demonstrated widespread occurrence of STX in the Alaskan marine environment across multiple trophic levels. Additionally, STX has been implicated as a possible factor in at least one recent seabird die-off event. These findings suggest that HABs present a hazard to seabirds and other marine consumers and warrant additional research.

# WIDESPREAD MORTALITY OF GULLS, TERNS, AND AUKLETS IN THE UNITED STATES LINKED TO THE BACTERIUM BISGAARD TAXON 40

Jeff Lorch<sup>1</sup>, Jason Ladner<sup>2</sup>, Barbara Bodenstein<sup>1</sup>, Susan Knowles<sup>1</sup>, Andrew Hubble<sup>3</sup>, Brenda Berlowski-Zier<sup>1</sup>, Gustavo Palacios<sup>4</sup>

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#### Abstract

In 2016, the U.S. Geological Survey-National Wildlife Health Center (NWHC) investigated mortality events involving gulls, terns, and auklets in which the Pasteurella-like bacterium Bisgaard taxon 40 (BT40) was identified as the causative agent. Bisgaard taxon 40 had been previously described from a respiratory tract lesion of a gull in Scotland, but the bacterium has not otherwise been reported as a pathogen. Mortality events linked to BT40 in 2016 were widespread, occurring on the East Coast, West Coast, and Great Lakes regions of the U.S. We examined data for mortality events involving Charadriiformes from 1998-2016 for which samples were submitted to NWHC for diagnostic evaluation. Although the NWHC had isolated BT40 from birds as early as 1999, the bacterium was considered an incidental finding and was not attributed to mortality until 2016. In that year, 33% of Charadriiformes mortality events were linked to BT40. To better understand the emergence of these infections, we screened oral and nasal swab samples from 48 apparently healthy gulls for BT40. Isolates of BT40 were recovered from 5 (10%) of the apparently healthy gulls. This, combined with the historical isolates obtained from birds that died of other causes, suggested that BT40 may be part of the normal flora of some birds. We next conducted whole genome sequencing analysis of 27 BT40 isolates. Thirteen of the isolates originated from birds that had died of BT40 infections, and these formed a distinct clade and possessed unique genes, indicating an epidemiological link between most of the mortality events. These data suggest that specific virulent strains of BT40 may have been responsible for the outbreak that occurred in 2016. The mechanisms responsible for the sudden appearance and spread of the virulent strain of BT40 in the U.S. are unknown, and further work is underway to better understand these emerging infections.

# CHARACTERIZATION OF A NOVEL BUNYAVIRUS ISOLATED FROM MORIBUND FARMED AND FREE-RANGING FRESHWATER TURTLES IN FLORIDA

Thomas B. Waltzek¹, Brian A. Stacy², Robert Ossiboff³, Nicole Stacy³, William Fraser⁴, Annie Yan⁴, Shipra Mohan⁴, Thais Rodrigues¹, Kuttichantran Subramaniam¹, Veronica Guzman-Vargas⁵, Lisa Shender⁵

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# Abstract

In 2006, increased mortality was observed on a Florida farm rearing softshell turtles (*Apalone ferox*; ST). A moribund ST was submitted to the Bronson Animal Disease Diagnostic Laboratory in Kissimmee, FL for diagnostic evaluation. Tissue homogenates inoculated onto confluent monolayers of Terrapene Heart (TH-1) cells resulted in extensive cytopathic effects (CPE). The supernatant was diluted and passaged onto fresh TH-1 cells with the same CPE observed. The supernatant was processed for negative stain electron microscopy and abundant spherical virus particles displaying prominent surface projections were observed.

An infected TH-1 culture was shipped to the University of Florida's Wildlife and Aquatic Veterinary Disease Laboratory in Gainesville, FL for genetic characterization. An RNA extract from the infected culture was used to generate a cDNA library for sequencing on a MiSeq sequencer. BLASTX searches of assembled contigs identified the tripartite genome of a novel bunyavirus including the viral genes encoding the RNA-dependent RNA polymerase (RdRp), glycoproteins, non-structural protein, and nucleoprotein. PCR confirmed the three turtle bunyavirus (TBV) segments were amplifiable in infected TH-1 cultures, but not in the TH-1 cell line. A PCR targeting the TBV RdRp was developed to screen freshwater turtle samples collected by staff of the Florida Fish and Wildlife Conservation Commission as part of an ongoing epizootic (2018-19). 7/7 STs and 2/3 peninsular cooters (*Pseudemys peninsularis*; PC) tested positive (35/36 and 4/8 tissue samples, respectively). Three additional STs sampled from unrelated cases tested negative by PCR (0/7) and the aforementioned PCR-negative (0/4) PC was determined to have died from trauma. We plan to develop a RNAscope in situ hybridization assay and a TaqMan quantitative PCR assay to assist in elucidating the tissue distribution, viral load, and pathogenesis of the TBV. An experimental challenge study will be conducted to determine the role of the TBV in disease of freshwater turtles.

# DISTRIBUTION AND PATHOLOGY OF A FRESHWATER TURTLE MORTALITY EVENT IN FLORIDA CAUSED BY A NOVEL BUNYAVIRUS

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#### Abstract

In March 2018, the Florida Fish and Wildlife Conservation Commission (FWC) began receiving reports of sick and dead Florida softshell turtles (Apalone ferox) and peninsula cooters (Pseudemys peninsularis) in the southern region of the St. Johns River (SJR). Softshell turtles were lethargic and abnormally docile. Cooters exhibited an inability to submerge. From March through July, at least 170 sick or dead turtles were recorded via FWC surveys and verified public reports. The outbreak spread approximately 250 km downstream and subsided by mid-July. However, in January 2019 mortalities resurged in the SJR headwaters, suggesting a possible seasonal component. Fifteen softshells and three cooters from the SJR were collected for necropsy and laboratory evaluation as of late February 2019. Ulcers and exudative plaques of the oral cavity, cloaca, and phallus were the most consistently observed gross lesions. However, PCR assays for iridoviruses and herpesviruses, common causes of such lesions, were negative. Histopathology revealed septic emboli, vasculitis, heterophilic and histiocytic meningoencephalitis, and multiorgan necrosis. Lesions included prominent infiltrates of degenerate macrophages. Suspected viral intracytoplasmic inclusions were identified within leukocytes in blood films and tissue imprints. These findings, along with the detection of secondary pathogens (e.g. mycobacteriosis, fungal colitis), led to the hypothesis that turtles were immunosuppressed due to an underlying viral infection. A novel bunyavirus was subsequently found based on next generation sequencing and reverse transcriptase PCR of tissues from multiple affected turtles. In addition, the virus was found to be genetically identical to a previously unreported virus from a 2007 farmed softshell turtle outbreak. Further studies are warranted to learn how the virus is transmitted, to document its spread and long-term impact on softshell turtle and cooter populations, and to determine whether the virus is pathogenic to other chelonian species, especially those that are imperiled.

#### THIAMINE DEFICIENCY IN NATURE AND ITS BIOLOGICAL CONSEQUENCES

Torsten Mörner

National Veterinary Institute

## Abstract

Thiamine (vitamin B1) is a water-soluble vitamin needed in all living cells, and as such it is a possible target for toxic action by environmental disturbances. Most animals acquire thiamine through their diet, so thiamine deficiency can arise if food is thiamine deficient. Production of thiaminase in prey species has been suggested as the cause of thiamine deficiency, but recent studies indicate that thiaminase is not the only cause of thiamine deficiency. Although thiamine deficiency is ultimately lethal, it also has a number of preceding sub-lethal health effects, such as reduced feeding, memory and learning disturbances, immunosuppression, damage to the blood-brain barrier, neurological disorders, and altered carbohydrate, protein, and lipid metabolism. During the last few decades, thiamine deficiency has been reported to occur in wildlife, such as: bivalves (Mytilussp.); fish such as Salmonines, European and American eels (Anguillaspp.) and also recently in Baltic cod (Gadus morhua); reptiles (American alligator (Alligator mississippiensis)), and birds (herring gulls (Larus argentatus), eider ducks (Somateria mollissima) and starlings (Sturnus vulgaris) in USA, Sweden and several other areas in North America and Europe. The lack of thiamine in wild animals leads to reproductive disorders, declining populations, impaired health, orientation disorders and behavioural changes. In a recent review, 24 researchers listed 117 threats to global biodiversity: thiamine deficiency in wildlife is recognized as one of these serious threats. Our recent studies also show that thiamine deficiency occurs in ruminants like roe deer (Capreolus capreolus), fallow deer (Dama dama), moose (Alces alces), and also in domestic animals like sheep, and in insects (domestic and wild bees, Apissp.). Further studies need to be carried out to determine the root cause of the thiamine deficiency and thus better understand in what way it affects animal health and behaviour and by that causing problem in the ecosystem, including alarming population declines.

# DISTRIBUTION OF CRIMEAN-CONGO HEMORRHAGIC FEVER IN THE IBERIAN PENINSULA IN RED DEER (CERVUS ELAPHUS) POPULATIONS

Francisco Ruiz-Fons<sup>1</sup>, Miriam Andrada Sas<sup>2</sup>, Ignacio García-Bocanegra<sup>3</sup>, Isabel García Fernandez de Mera<sup>1</sup>, Isolde Schuster<sup>2</sup>, Sven Reiche<sup>2</sup>, Marc Mertens<sup>2</sup>, David Cano-Terriza<sup>2</sup>, Laia Casades-Martí<sup>1</sup>, Saúl Jiménez-Ruiz<sup>3</sup>, Martin Groschup<sup>2</sup>

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## Abstract

Crimean-Congo hemorrhagic fever (CCHF) is an emerging zoonotic vector-borne disease in the Iberian Peninsula and two fatal cases have been reported in 2016 and 2018 in Spain after tick bites in relation to outdoor/hunting activities. CCHF virus is mainly transmitted by ticks of the genus Hyalomma and the red deer (Cervus elaphus) is a major host for Hyalomma ticks in Iberia. Therefore, the major goal of this study was to estimate the spatiotemporal distribution of CCHFv in the populations of red deer in the Iberian Peninsula as a first stage to depict the real spatial spread of the virus. To approach this study, 1,444 sera collected from wild free-roaming Iberian red deer in 64 Spanish and 5 Portuguese populations, within the distribution range of the species, were analyzed by a competitive ELISA using a His-tagged recombinant N protein of CCHFv strain Kosovo Hoti as antigen. A total of 373 sera (25.8%; 95%CI: 23.6-28.2) were positive and 43 of the 69 (62.3%) surveyed locations had at least one seropositive individual. In eight locations over 50% of the red deer were exposed whereas seropositivity ranged between 30 and 50% in 11 additional locations. The highest rates were observed in the southwestern quarter of Iberia where Hyalomma ticks, large ungulates and extensively raised livestock are abundant and coexist. Some few positive samples were also found in northern Spain. Our results point to a widespread distribution of CCHFv in Spain in spite of the low number of human cases reported to date. These results indicate that hunters may have an increased risk of acquiring CCHFv infections. Therefore, wild ungulate-tick relationships should be further explored in Iberia in relation to CCHFv.

# WILDLIFE AS SENTINELS FOR EMERGING ZOONOTIC DISEASES: HEPATITIS E AND TICK-BORNE ENCEPHALITIS IN CERVIDS IN NORWAY

Carlos das Neves<sup>1</sup>, Knut Madslien<sup>1</sup>, Katrine Mørk Paulsen<sup>2</sup>, Åshild Kristine Andreassen<sup>2</sup>, Carlos Sacristán Yagüe<sup>1</sup>

<sup>1</sup>Norwegian Veterinary Institute, <sup>2</sup>Norwegian Institute of Public Health

## Abstract

The knowledge that up to 70% of emerging diseases around the world arise from wild animals raises the importance of mapping how zoonotic pathogens spread in an ecosystem before they reach humans or e.g. production animals. In Norway, we have conducted two serological studies in cervids targeting two zoonotic pathogens that are occasionally reported in the country: hepatitis E (HEV) in pigs and humans, and tick-borne encephalitis virus (TBEV) in ticks and humans. Given the current northward range expansion of wild boars and ticks, the identification of these pathogens in cervids, which are abundant all over Norway, may serve as a sentinel / early warning systems.

Serum samples from 286 moose, 148 roe deer, 140 red deer and 83 reindeer from all over Norway were collected and screened by 2 different serological assays for antibodies against TBEV. The overall seroprevalence was 4.3%. The highest number of TBE seropositive cervids was found in Eastern Norway, but seropositive cervids were also detected in Central Norway in areas where the virus has never been reported in humans. Antibodies against TBEV were present in 9.4% of the analysed samples from moose, 1.4% in red deer, 0.7% in roe deer and 0% in wild reindeer. Serum samples from 165 moose, 86 roe deer, 186 wild reindeer and 177 red deer from all over Norway were screened for antibodies against HEV. The overall seroprevalence was 13,4%. The highest number of seropositive was found in wild reindeer (25,3%) and moose (19,4%).

Results clearly show that both HEV and TBEV are circulating in wild cervids in Norway, including in areas where these pathogens have not yet been reported in humans or other animals. This clearly demonstrates the importance of wild animals as sentinel species for understanding the epidemiology of emerging diseases and detecting them as early as possible.

# EMERGENCE & MOLECULAR CHARACTERIZATION OF PIGEON PARAMYXOVIRUS-1 IN FREE-RANGING COLUMBIDS IN CALIFORNIA

Krysta Rogers¹, Asli Mete², Hon Ip³, Mia Torchetti⁴, Mary Killian⁴, Beate Crossley²

<sup>1</sup>Wildlife Investigations Lab, California Department of Fish and Wildlife, <sup>2</sup>California Health and Food Safety Laboratory, University of California, Davis, <sup>3</sup>U.S. Geological Survey, National Wildlife Health Center, <sup>4</sup>National Veterinary Services Laboratory, United States Department of Agriculture

#### Abstract

Eurasian collared doves (Streptopelia decaocto) were introduced into Florida in the 1980s and have since established populations throughout the continental United States. Pigeon Paramyxovirus-1 (PPMV-1), a species-adapted genotype VI Avian Avulavirus-1, causes periodic outbreaks among columbid species. Outbreaks in collard doves have been reported across the U.S. since 2001, and sometimes involve native species of doves. In California, mortality events involving collared doves were first documented in Riverside County in 2014. Affected doves displayed neurological signs including torticollis and partial paralysis. Encephalitis and renal necrosis were identified on post-mortem exam. Pigeon Paramyxovirus-1 was confirmed by PCR and sequence analysis from oral and cloacal swabs. Between 2015 and 2018, subsequent outbreaks in collared doves have been confirmed or suspected in 20 additional counties from southern to northern California. In 2017, PPMV-1 was detected by PCR from formalin-fixed paraffin-embedded tissues, after the initial PCR from swabs failed to detect the virus, in one native mourning dove (Zenaida macroura) found dead during an outbreak in collared doves. Additionally, PPMV-1 was confirmed in non-native rock pigeons (Columba livia) in a separate outbreak in 2016. Molecular sequencing of the fusion protein of isolates collected from collared doves in 2014 and 2017 identified two distinct subgenotypes, VIa and VIn, co-circulating in California. Subgenotype VIn has been primarily isolated from collared doves in the southern U.S., while VIa has been isolated from mixed avian species in the northeastern U.S., suggesting two independent introductions into California. While populations of collared doves and rock pigeons are not expected to be impacted by this disease, PPMV-1 may pose a threat to already declining populations of native columbids. This potential threat could be assessed by monitoring native and non-native columbids for PPMV-1. Previous experience suggests that surveillance in native columbids may require use of alternative testing methods to ensure virus detection.

# NOVEL ORTHOREOVIRUS ASSOCIATED WITH EPIZOOTIC NECROTIZING ENTERITIS AND SPLENIC NECROSIS IN AMERICAN CROWS (CORVUS BRACHYRHYNCHOS)

María Forzán<sup>1</sup>, Randall Renshaw<sup>1</sup>, Elizabeth Bunting<sup>1</sup>, Elizabeth Buckles<sup>1</sup>, JOseph Okoniewski<sup>2</sup>, Kevin Hynes<sup>2</sup>, Richalice Melendez<sup>1</sup>, Ashley Ableman<sup>2</sup>, Melissa Laverack<sup>1</sup>, Melissa Fadden<sup>1</sup>, Akbar Dastjerdi<sup>3</sup>, Krysten Schuler<sup>1</sup>, Edward Dubovi<sup>1</sup>

<sup>1</sup>Cornell University, <sup>2</sup>New York Department of Environmental Conservation, <sup>3</sup>Animal and Plant Health Agency

# Abstract

Epizootic mortalities in American crows (Corvus brachyrhynchos) during the winter months have been recorded in North America for almost two decades and referred to as "winter mortality of crows". Most common postmortem findings include necrotizing enteritis, colitis and fibrinous splenic necrosis. These signs are proposed to be due to infection with a Reovirus sp. Our objectives were to characterize the pathology and seasonality of the epizootics in New York State (NYS), confirm the causative role of an Orthoreovirus sp and determine its phylogeny. Based on our proposed case definition for reovirosis, we examined case data collected by the NYS Wildlife Health Program (NYS WHP) for a 16-year period of time. A total of 558 cases of reovirosis were recorded between 2001-2017. Reovirosis had a clear seasonal presentation: cases occurred almost exclusively in winter months (71% in December-January). Detailed data from a two-year period (2016-2017) demonstrated that reovirosis caused up to 70% of all recorded crow deaths during epizootic months. Crows with positive orthoreovirus isolation from spleen or intestine were 32 times more likely to die with characteristic histologic lesions of enteritis/enterocolitis and splenic necrosis than crows with negative isolation results. An in situ hybridization probe specific to virus isolated from NYS crow reovirosis cases demonstrated a direct association between viral presence and characteristic histopathologic lesions. Sigma C (capsid protein) sequences of isolates from NYS crows showed high homology with Tvärminne avian virus, recently proposed as a novel Corvus orthoreovirus clade, and only distantly related to the avian orthoreovirus clade. Our study indicates that a novel orthoreovirus is the cause of "winter mortality", or reovirosis, of American crows and places the NYS isolates in the newly proposed genus of Corvid orthoreovirus.

# ANTICOAGULANT RODENTICIDE EXPOSURE IN BALD EAGLES (HALIAEETUS LEUCOCEPHALUS) AND GOLDEN EAGLES (AQUILA CHRYSAETOS) IN THE UNITED STATES

Kevin Niedringhaus<sup>1</sup>, Nicole Nemeth<sup>2</sup>, Samantha Gibbs<sup>3</sup>, Jared Zimmerman<sup>4</sup>, Lisa Shender<sup>4</sup>, Kate Slankard<sup>5</sup>, Heather Fenton<sup>6</sup>, Charlie Bahnson<sup>7</sup>, Martha Frances-Dalton<sup>2</sup>, Betsy Elsmo<sup>8</sup>, Bob Poppenga<sup>9</sup>, Mark Ruder<sup>10</sup>

<sup>1</sup>Southeastern Cooperative Wildlife Disease Study, University of Georgia, <sup>2</sup>University of Georgia, <sup>3</sup>United States Fish and Wildlife Service, <sup>4</sup>Florida Fish and Wildlife Conservation Comission, <sup>5</sup>Kentucky Department of Fish and Wildlife Resources, <sup>6</sup>Department of Environment and Natural Resources, Goverment of Northwest Territories, <sup>7</sup>North Dakota Game and Fish Department, <sup>8</sup>Wisconsin Veterinary Diagnostic Laboratory, <sup>9</sup>CAHFS Laboratory, University of California, Davis, <sup>10</sup>Southeastern Cooperative Wildlife Disease Study, University of Georgia

## Abstract

Bald eagles (Haliaeetus leucocephalus) and golden eagles (Aquila chrysaetos canadensis) are broadly distributed in North America and play important ecosystem functions as predators and scavengers. Once critically endangered, the recovery of bald eagles represents a conservation success story after changes to management strategies, including regulatory protections and other actions, such as banning use of the pesticide dichlorodiphenyltrichloroethane (DDT). However, eagles and other wildlife are exposed to a variety of toxic compounds in the environment that could have population impacts. Few studies have examined anticoagulant rodenticide (AR) exposure in bald and golden eagles. The objective of this study was to determine the types and quantities of ARs in eagle carcasses submitted for diagnostic examination to the Southeastern Cooperative Wildlife Disease Study. Necropsy case submission records from bald eagles (n=244) and golden eagles (n=56) received from 2014-2018 were reviewed. Anticoagulant rodenticide screens were performed on livers from 114 bald eagles and 17 golden eagles and one or more ARs were reported at detectable levels (i.e., exposure) in 109 cases (83.2%). The diagnosis of AR toxicosis was made when gross or microscopic hemorrhage was observed without evidence of other potential causes (e.g., trauma). Overall, AR toxicosis was determined to be the primary cause of mortality in 13 eagles (4.3%). The percentages of AR exposure in eagles was high (83.3% in bald eagles and 76.5% in golden eagles) compared to those reported for raptors in other studies, but the proportion of AR-associated mortality was lower (4.3%). The most frequently detected compounds were brodifacoum (80%) and bromadiolone (25%). These results suggest that many ARs are widespread in the environment and eagles are commonly exposed, likely through ingestion of food sources. This study highlights the need for additional research to assess potential individual and population-level impacts of AR exposure in eagles and other raptors.

# FURTHER INQUIRY INTO THE ETIOLOGY OF HOOF DISEASE IN ELK USING BACTERIAL METAGENOMICS ANALYSIS

Margaret Wild¹, Kim Chiok¹, Devendra Shah¹, Jennifer Wilson-Welder², Kyle Taylor¹, Kristin Mansfield³, Sushan Han⁴

<sup>1</sup>Washington State University, <sup>2</sup>USDA/ARS, <sup>3</sup>Washington Department of Fish and Wildlife, <sup>4</sup>Colorado State University

# Abstract

Cases of limping elk (Cervus elaphus) exhibiting characteristic hoof lesions reported to the Washington Department of Fish and Wildlife increased markedly in southwest Washington in 2008. Currently, the disease has been documented in localized areas across western and southern Washington, northern Oregon, and western Idaho. Lesions are characterized by deformed, overgrown, broken or sloughed hooves, often with severe sole ulcers, extensive laminar necrosis, and pedal osteomyelitis. Spirochetes are observed on histology. In previous studies, Treponema spp. similar to those reported in digital dermatitis of cattle and sheep were isolated sporadically from lesions, and the presence of treponemes in tissues was supported by immunohistochemistry and PCR. Thus, the disease is currently diagnosed as treponeme-associated hoof disease (TAHD) based on gross lesions and histological presence of spirochetes. While treponeme-associated, additional investigation is warranted to determine the suite of organisms that might contribute to etiology. For example, digital dermatitis in livestock is generally considered to be a polymicrobial disease and predictable changes in the bacterial microbiota have been described through the progression of hoof lesions in cattle. We hypothesize a similar process may occur in elk. In a pilot study, we compared the bacterial (16S rDNA) metagenomes in biopsies collected postmortem from healthy (n=4) and diseased (n=4) elk feet. The bacterial diversity was reduced in the diseased state when compared to control samples. Results supported treponeme association; Spirochetes (Treponema spp.), as well as Tenericutes (Mycoplasma spp.) and Fusobacteria (Fusobacterium spp.), were the most predominant bacteria Phyla associated with lesions. Proteobacteria (Halomonas spp.) were overrepresented in control samples. Ongoing studies are evaluating larger numbers of samples collected from different lesion grades and geographically distant from the area of initial disease detection. Findings are critical for epidemiological investigation, developing a disease challenge model, and identifying potentially effective mitigation actions.

# THE WILD WORLD OF GUINEA WORMS: TRANSMISSION DYNAMICS AND PHYLOGENETICS OF AFRICAN AND NORTH AMERICAN GUINEA WORMS IN WILDLIFE, DOMESTIC ANIMALS, AND HUMANS

Christopher Cleveland<sup>1</sup>, Mark Eberhard<sup>2</sup>, Alec Thompson<sup>1</sup>, Kayla Garrett<sup>1</sup>, Erin Box<sup>1</sup>, Hubert Zirimwabagabo<sup>3</sup>, Mario Romero<sup>3</sup>, Adam Weiss<sup>3</sup>, Ernesto Ruiz-tiben<sup>3</sup>, Michael Yabsley<sup>1</sup>

<sup>1</sup>University of Georgia/Dept. of Population Health/Southeastern Cooperative Wildlife Disease Study, <sup>2</sup>Centers for Disease Control and Prevention/Retired, <sup>3</sup>The Carter Center

#### Abstract

Cases of limping elk (Cervus elaphus) exhibiting characteristic hoof lesions reported to the Washington Department of Fish and Wildlife increased markedly in southwest Washington in 2008. Currently, the disease has been documented in localized areas across western and southern Washington, northern Oregon, and western Idaho. Lesions are characterized by deformed, overgrown, broken or sloughed hooves, often with severe sole ulcers, extensive laminar necrosis, and pedal osteomyelitis. Spirochetes are observed on histology. In previous studies, Treponema spp. similar to those reported in digital dermatitis of cattle and sheep were isolated sporadically from lesions, and the presence of treponemes in tissues was supported by immunohistochemistry and PCR. Thus, the disease is currently diagnosed as treponeme-associated hoof disease (TAHD) based on gross lesions and histological presence of spirochetes. While treponeme-associated, additional investigation is warranted to determine the suite of organisms that might contribute to etiology. For example, digital dermatitis in livestock is generally considered to be a polymicrobial disease and predictable changes in the bacterial microbiota have been described through the progression of hoof lesions in cattle. We hypothesize a similar process may occur in elk. In a pilot study, we compared the bacterial (16S rDNA) metagenomes in biopsies collected postmortem from healthy (n=4) and diseased (n=4) elk feet. The bacterial diversity was reduced in the diseased state when compared to control samples. Results supported treponeme association; Spirochetes (Treponema spp.), as well as Tenericutes (Mycoplasma spp.) and Fusobacteria (Fusobacterium spp.), were the most predominant bacteria Phyla associated with lesions. Proteobacteria (Halomonas spp.) were overrepresented in control samples. Ongoing studies are evaluating larger numbers of samples collected from different lesion grades and geographically distant from the area of initial disease detection. Findings are critical for epidemiological investigation, developing a disease challenge model, and identifying potentially effective mitigation actions.

# RISK ASSESSMENT FOR THE INCURSION AND ESTABLISHMENT OF ORBIVIRUSES IN ONTARIO, CANADA: SEROSURVEILLANCE IN WILDLIFE AND LIVESTOCK AND CHARACTERIZATION OF CURRENT VECTOR COMPOSITION AND DISTRIBUTION

Samantha E. Allen<sup>1</sup>, Claire Jardine<sup>1</sup>, Nicole Colucci<sup>2</sup>, Tara Furukawa-Stoffer<sup>2</sup>, Aruna Ambagala<sup>2</sup>, Kathleen Hooper-McGrevy<sup>2</sup>, Mark Ruder<sup>3</sup>, Nicole Nemeth<sup>3</sup>

<sup>1</sup>University of Guelph, <sup>2</sup>Canadian Food Inspection Agency, <sup>3</sup>University of Georgia/Dept. of Population Health/ Southeastern Cooperative Wildlife Disease Study

# Abstract

Epizootic hemorrhagic disease viruses (EHDV) and bluetongue viruses (BTV) are midge-borne orbiviruses presenting an imminent threat to Ontario's wildlife and livestock populations. These viruses are spreading northward in North America, which may be facilitated by changing climatic conditions. Recent detection of BTV antibody-positive cattle and Culicoides sonorensis in the province suggest that Ontario is at risk for the establishment of EHDV and BTV. Ontario ruminants are immunologically naïve to these viruses; thus, their introduction may negatively impact wild cervid and livestock populations through morbidity and mortality. We sought to characterize Culicoides spp. biology and assess for recent or ongoing transmission of EHDV and BTV in wild cervids and livestock in Ontario for two spring-summer field seasons (2017-2018).

During two field seasons (2017-18), CDC-type LED light traps were placed on farms and in natural areas across southwestern Ontario, and Culicoides spp. midges were taxonomically and molecularly identified. Blood from wild cervids and livestock were assessed for antibodies to EHDV and BTV by ELISA and subsequent virus neutralization assay for serotyping.

During the 2017 field season, we collected 19,126 Culicoides spp., including individuals of at least eight species: C. sonorensis, C. variipennis, C. biguttatus, C. crepuscularis, C. stellifer, C. travisi, C. haematopotus and C. venustus, with the most commonly identified species being in the subgenus Avaritia. Thus far, in 2018, we have collected 7,604 Culicoides spp., with C. biguttatus being the most commonly identified. Regional, temporal, and climatic patterns in vector proportions and distribution and seroprevalence data will be statistically analyzed. Furthermore, in 2017, we identified two white-tailed deer that died of EHDV (serotype 2) and 15 cattle seropositive for EHDV-2, these represent the first EHDV detections in Ontario, Canada. Collectively, these results will help improve current policies and practices for safeguarding Ontario wild cervid and livestock populations.

# IMPACTS OF PRESCRIBED FIRE AND FOREST THINNING ON TICK POPULATIONS AND PREVALENCE OF TICK-BORNE DISEASES IN A SOUTHEASTERN MIXED PINE FOREST

Brent Newman<sup>1</sup>, William Sutton<sup>1</sup>, Abelardo Moncayo<sup>2</sup>

<sup>1</sup>Tennessee State University, <sup>2</sup>Tennessee Department of Health

#### Abstract

Landscape disturbance in the form of forest management can greatly alter habitat and climatic conditions causing shifts in vector-borne disease pathways. However, the extent that tick populations and prevalence of tick-borne diseases are impacted by forest management in the southeastern United States remains poorly understood. From June to August 2016 and 2017, we evaluated the effects of prescribed fire and forest thinning on tick species diversity and abundance as well as tick-borne disease prevalence at 18 management stands within the William B. Bankhead National Forest, Alabama. Ticks were sampled at 6 randomly generated coordinates at each research stand (n= 216 sampling events over 2 years) via drag sampling. Using a qPCR approach, nymph and adult ticks were tested for Borrelia lonestari, Heartland Virus, and Rickettsia spp. Our results suggest heavy thinning may lead to a significant increase in tick populations while both heavy and light thinning coupled with prescribed fire application may significantly decrease tick populations. Tick species composition was unaffected by management as Lone star ticks (Amblyomma americanum) were the most commonly encountered species in 2016 and 2017 (93% and 94% of total specimens, respectively). Heartland Virus was not detected, however, Rickettsia spp. and Borrelia lonestari were detected from collected ticks at both nymph and adult life stages throughout all research stands with no significant differences in disease prevalence based on forest management technique observed. In conclusion, our data suggest prescribed fire coupled with forest thinning may be an effective option for managing tick populations which may decrease opportunity for tick-borne disease transmission in southeastern forest ecosystems.

# ASSESSMENT OF BIODIVERSITY RELATIONSHIPS IN TURTLE AND LEECH PARASITE-HOST ASSEMBLAGES IN MIDDLE TENNESSEE WETLANDS ACROSS A DISTURBANCE GRADIENT

Laura Horton<sup>1</sup>, William Sutton<sup>1</sup>

<sup>1</sup>Tennessee State University

#### Abstract

We are currently experiencing a global biodiversity crisis, and ectotherms represent the organismal group that faces the largest threat of species diversity loss due to global change. Prior research has established clear links between decreased reptile biodiversity in degraded or disturbed habitats, specifically with chelonian groups. There are also negative impacts associated with high parasite loads on hosts, and previous studies have found parasite loads increase with habitat disturbance, thus we aimed to assess if a seasonal trend or trend by habitat disturbance exists for varying leech loads on wetland turtles. To better understand the effects of human influence on wetland turtles, we sampled 19 wetlands in Middle Tennessee over the months of June through October of 2018. Wetlands were scored into three categories of anthropogenic impact; reference sites (low disturbance; n=4), intermediate sites (medium disturbance; n=8), and degraded sites (high disturbance; n=7) using the National Land Cover Database and on-ground wetland rapid assessment scores. Each wetland was sampled on four occasions per site, with 6 baited hoop-net traps deployed for 24 hours per each sampling event. We captured 922 turtles from four species, and 917 leeches from three species. We saw significantly higher parasite loads at degraded wetlands than at reference wetlands. We also saw a seasonal pattern in leech abundance which followed established data on the leech life cycle, with leech abundance highest in mid-summer months. The results from this study support other studies of its kind which found higher risk of parasitism in more degraded environments, as well as prior publications indicating the seasonal trends in leech abundance on turtles. Collectively, the interpretation of these data can be used to understand how the effects of anthropogenic disturbance affect wetland turtle-leech communities.

## PITFALLS OF TELEMETRY-DERIVED CONTACT NETWORKS OF WILDLIFE

Marie Gilbertson<sup>1</sup>, Lauren White<sup>2</sup>, Meggan Craft<sup>1</sup>

<sup>1</sup>University of Minnesota, <sup>2</sup>National Socio-Environmental Synthesis Center

# Abstract

Contact network modeling of infectious disease in wildlife can reveal traits or individuals critical to pathogen transmission and help inform disease management strategies. However, estimates of contact between animals are notoriously difficult to acquire. Researchers commonly use telemetry technologies to identify animal interactions; such data, however, may have different sampling intervals and often captures a small subset of the population. The objective of this study was to understand the consequences of telemetry sampling on our ability to detect contacts and estimates of network structure. We simulated individual movement trajectories for wildlife populations using a home range-like movement model, creating full location datasets and corresponding "complete" networks. To mimic telemetry data, we created "sample" networks by subsampling the population (10-100% of individuals) and sampling interval (every minute to every three days). We varied the definition of contact for sample networks, using either spatio-temporal or home range overlap. To compare complete and sample networks, we calculated six network metrics known to be important for disease transmission and assessed mean ranked correlation coefficients between complete and sample network metrics. Telemetry sampling severely reduced our ability to calculate the network metrics of betweenness and clustering coefficient, with less impact on other metrics. In populations with infrequent interactions, however, high intensity sampling may still be necessary. Defining contact in terms of spatial overlap generally resulted in overly connected networks, but in some instances, could optimize otherwise coarse telemetry data. By synthesizing movement ecology, computational, and disease ecology approaches, we characterized trade-offs important for determining the utility of wildlife telemetry data beyond ecological studies of individual movement, and found careful use of telemetry data has the potential to inform network models. Thus, with informed application of telemetry data, we can make significant advances in leveraging its use for better understanding and management of wildlife infectious disease.

#### FRUIT BATS IN FLIGHT: HOW THEY INFORM CONSERVATION AND DISEASE SURVEILLANCE IN TANZANIA

Nistara Randhawa<sup>1,2</sup>, Brian Bird<sup>1,2</sup>, Elizabeth Van Wormer³, Zikankuba Sijali⁴, Chris Kilonzo<sup>1,2</sup>, Alphonce Msigwa⁴, Abel Ekiri⁵, Aziza Samson⁴, David Wolking<sup>1,2</sup>, Woutrina Smith<sup>1,2</sup>, Beatriz Martínez-López⁵, Rudovick Kazwala⁴, Jonna A.K. Mazet<sup>1,2</sup>

<sup>1</sup>One Health Institute, School of Veterinary Medicine, University of California, Davis, <sup>2</sup>Karen C. Drayer Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, <sup>3</sup>School of Veterinary Medicine & Biomedical Sciences, School of Natural Resources, University of Nebraska, Lincoln, <sup>4</sup>Sokoine University of Agriculture, <sup>5</sup>School of Veterinary Medicine, University of Surrey, <sup>6</sup>Center for Animal Disease Modeling and Surveillance, School of Veterinary Medicine, University of California, Davis

## Abstract

**Background**: Many ecologically important plants are pollinated or have their seeds dispersed by fruit bats, the African straw-colored fruit bats (Eidolon helvum) being one of the most widely distributed of them. Their ability to fly long distances makes them essential for connecting plant populations across fragmented landscapes. Bats are also reservoirs of viruses pathogenic to humans, such as some coronaviruses and Ebola, Marburg, and Hendra.

**Objectives**: Track the movements of E. helvum in Tanzania to shed light on their foraging patterns and identify factors informing their conservation and risks for disease transmission to other species. Methods: GPS loggers were deployed on 25 bats captured in the Morogoro Municipal and Kilombero District near the Udzungwa Mountains of Tanzania. Foraging and additional roosting sites were determined from bat movement data and characterized according to their proximity to urban and protected areas. Sites for additional environmental surveillance via camera traps were also identified from tracking data.

**Results**: Tracking data revealed variability between individual bat movements and a fidelity to foraging areas. While the majority of their foraging locations were in or near urban areas, bats also fed in protected areas, Udzungwa Mountains National Park (UMNP) being the most frequented. Camera traps in fruit orchards frequented by tracked bats showed the presence of multiple species, including a Vervet monkey (Chlorocebus pygerythrus), at these bat foraging sites.

**Conclusions**: E. helvum plays an important role in the pollination and seed dispersal of plants within both protected and urban areas in Tanzania; the conservation of bats and protected areas is thus interdependent. Their movement patterns also suggest the possibility of contact among bats, non-human primates, livestock, and humans in bat foraging areas near human dwellings and in horticulture, as well as an opportunity for viral sharing to occur between bats and other wild species in protected areas.

# **STUDENT POSTERS**

# PREVALENCE AND TRANSMISSION OF CRYPTOSPORIDIUM AND GIARDIA INFECTIONS AMONGST LEMURS, HUMANS, DOMESTIC MAMMALS AND INVASIVE RATS IN TSINJOARIVO, MADAGASCAR

Laurie Spencer<sup>1</sup>, Mitchell Irwin<sup>1</sup>

<sup>1</sup>Northern Illinois University

#### Abstract

Cryptosporidium and Giardia are ubiquitous enteric protozoan pathogens that infect humans, domestic animals, and wildlife worldwide. These pathogens cause gastroenteritis in their hosts, often being fatal in immunocompromised individuals. Both parasites are zoonotic and cross-species transmissions have occurred between humans, wildlife and domestic animals. Few studies have been conducted on the prevalence of Cryptosporidium and Giardia infections in Madagascar. Since protozoan parasites have not been studied in Tsinjoarivo, this project provides a baseline study of these pathogens in a cross-species sample of humans and other mammals in this area. Fecal samples were collected from two diurnal lemur species (Propithecus diadema and Hapalemur griseus), humans, domestic mammals (cattle, pigs and dogs), and invasive rats. The fecal samples were tested for Cryptosporidium and Giardia utilizing immunofluorescence assay. No lemurs were positive for Cryptosporidium or Giardia infection, but Cryptosporidium was found in humans (10%), cattle (20%), pigs (20%), dogs (15%) and invasive rats (38%), and Giardia was found in humans (10%), pigs (40%), dogs (29%) and invasive rats (53%). I assessed possible risk factors for humans using: age, sex, household size, gastrointestinal symptoms, frequency of forest entry, and contact with lemurs, domestic mammals and invasive rats. All infected human subjects were ≤13 years old, but no risk factors were statistically significant. Potential infection risk factors for domestic mammals were also examined (age and group size) with no significant results found. I detected coinfections of Cryptosporidium and Giardia in humans (6%), pigs (20%), dogs (15%) and invasive rats (33%). As human populations increase, human-wildlife encounters also increase, making it critical to understand this interface. The results of this study document zoonotic health concerns in Madagascar and can aid public health and conservation efforts by adding to our understanding of infection risks for sympatrically living humans, wildlife and domestic animals.

# DEVELOPMENT OF A DIAGNOSTIC ASSAY FOR DETECTION AND DIFFERENTIATION OF THEILERIA SPP. IN WHITE-TAILED DEER

Alec Thompson

University of Georgia/Dept. of Population Health/Southeastern Cooperative Wildlife Disease Study

## Abstract

The spread of non-native parasites with the movement of animals is a major concern for disease emergence and native species health/conservation. Haemaphysalis longicornis (Asian longhorned tick) is native to eastern Asia, but has become invasive in several countries including Japan, Australia, New Zealand, and now the United States. Within the established range, H. longicornis is a vector of the protozoan parasite, Theileria orientalis subtype Ikeda, which until recently was not known to occur in the United States. In 2017, clinical theileriosis caused by T. orientalis Ikeda was reported in a cattle herd in Virginia which was also infested with H. longicornis, but it is not known if H. longicornis transmitted T. orientalis to the cattle. Within the United States, white-tailed deer (WTD, Odocoileus virginianus) are infected with several genotypes of a Theileria sp. (often called T. cervi) that is distinct from T. orientalis. It is currently unknown if deer are susceptible to T. orientalis, a pathogen of agricultural concern. In this study, we developed a restriction fragment length polymorphism (RFLP) assay that can distinguish between Babesia spp., selected exotic Theileria spp., and the WTD Theileria sp. (although not the different genotypes [F, G1, and G2]). Using this assay, we tested cervid blood samples from the eastern United States and found 257 of 306 (84%) positive for the WTD Theileria. sp., 26 (8%) positive for Babesia spp., and 3 (<1%) for exotic Theileria spp. Twenty of the 264 samples had insufficient quantities of DNA to visualize RFLP results. Sequencing of 30 randomly selected samples confirmed RFLP results. Sequences of the three 'exotic' Theileria amplicons were >99% similar to T. ovis, a nonpathogenic ovine parasite native to Asia. Our data indicate that WTD Theileria. sp. infections are common and that WTD can possibly maintain a higher diversity of Theileria spp. than recognized.
# THE NEW RAT LUNGWORMS: THE OCCURRENCE OF PHYSALOPTERA HISPIDA AND A MASTOPHORUS SP. IN PULMONARY VESSELS OF THE HISPID COTTON RAT (SIGMODON HISPIDUS) FROM GEORGIA, U.S.A.

Alec Thompson<sup>1</sup>, Christopher Cleveland<sup>1</sup>, Troy Koser<sup>1</sup>, Seth Wyckoff<sup>1</sup>, Michael Yabsley<sup>1</sup>

<sup>1</sup>University of Georgia/Dept. of Population Health/Southeastern Cooperative Wildlife Disease Study

### Abstract

During 2017-2018, a survey for the rat lungworm, Angiostrongylus cantonensis (Nematoda: Metastrongyloidea) in rodents from Piedmont and Lower Coastal Plains physiographic regions of Georgia, U.S.A. was conducted. On four occasions, a single worm was recovered from the pulmonary vessels of a single cotton rat (Sigmodon hispidis). One of these worms was identified as a Physaloptera sp. and the remaining three as a Mastophorus sp. by morphology. Physaloptera (Nematoda: Physalopteroidea) and Mastophorus species (Nematoda: Spiruroidea) are stomach parasites of many wild and domestic animals. This is the first report of these species in the pulmonary vessels of a definitive host. To better characterize these parasites, representative specimens were collected from cotton rat stomachs and identified morphologically and molecularly. Based on partial cytochrome c oxidase subunit 1 (COI) gene sequences, Physaloptera hispida from stomachs were identical to the Physaloptera sp. from the pulmonary vessels. The COI sequences from the Mastophorus sp. from the stomach exhibited a higher degree of variability but confirmed that the pulmonary worms were the same Mastophorus species. Furthermore, sequences of Mastophorus from a coastal site clustered separately from a clade of Mastophorus sequences from cotton rats from a Piedmont site. Collectively, our data show that adult worms recovered from pulmonary vessels of cotton rats could be either Physaloptera or Mastophorus sp., indicating that these parasitic worms are not always restricted to the stomach and that worms from pulmonary vessels must be carefully examined to obtain a definitive diagnosis of A. cantonensis infection.

# ASSESSING THE EFFECTS OF COMPETITION AND SEASONALITY ON STRESS, IMMUNITY, AND NUMBER OF SCARS IN DEERMICE

Andreas Eleftheriou<sup>1</sup>, Amy Kuenzi<sup>2</sup>, Angela Luis<sup>1</sup>

<sup>1</sup>University of Montana, <sup>2</sup>Montana Tech of the University of Montana

### Abstract

Infectious wildlife diseases are becoming more common, causing population declines and species extinctions. Ecological and environmental stressors can influence disease spread in wildlife, through effects on parasite transmissibility (regulated by host immunity), and contact rates. Glucocorticoids (GCs) are hormones that mediate physiological and behavioral responses to stressors, and thus, can influence immunity and behavior in wildlife. Sin Nombre virus (SNV) is carried by deermice (Peromyscus maniculatus), and in western Montana grasslands, deermice compete with voles (Microtus spp.) and shrews (Sorex spp.). Because voles are dominant over deermice, they could increase SNV prevalence in deermice via stress-induced immunosuppression and/ or alteration in contact rates, while shrews may have a lesser effect. Our objectives were to investigate whether voles and/or shrews could increase SNV prevalence in deermice through changes in stress, immunity and/ or contact rates, and to examine seasonal changes in these same measures. We live-trapped small mammals over 2 years in western Montana grasslands. We evaluated deermice for scar numbers (proxy for contact rates), demography, and body condition scores (BCSs). Deermouse blood was evaluated for white blood cell counts/differentials, and SNV antibodies, and feces for fecal corticosterone metabolites (FCMs) to measure stress (baseline and stress-induced). Using mixed effect regression trees, we found that higher vole density was inconsistently associated with lower BCSs and scar numbers. Higher shrew density was consistently associated with lower stress-induced FCMs, but inconsistently with lower BCSs, and higher scar numbers. Neutrophil: lymphocyte (N:L) ratios were highest in spring/summer, and lowest in fall/winter. Due to low SNV prevalence, we could not evaluate effects on infection. Interspecific competition may influence disease spread via effects on chronic stress (i.e. lower stress-induced FCMs and BCSs), and scar numbers. Higher N:L ratios, suggestive of chronic stress, over spring/summer may provide an ideal time for SNV transmission. Our findings may extend to other diseases.

# ASSESSMENT OF THE STRESS RESPONSE IN DEERMICE: VALIDATION OF IMMUNOASSAYS FOR FECAL CORTICOSTERONE METABOLITES

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## Abstract

Anthropogenic factors that alter stress physiology in wildlife could affect disease dynamics in species that are important disease reservoirs, such as deermice (Peromyscus maniculatus). To understand how these factors influence disease dynamics, we need to develop robust stress physiology measures. Fecal cortisol/corticosterone metabolites (FCMs) are a non-invasive measure for evaluating stress physiology. However, immunoassays that measure FCMs need to be appropriately validated prior to their use, and factors that can influence FCMs, such as capture-induced stress, should be considered during their use. In the laboratory, we tested the suitability of three immunoassays (one corticosterone RIA, one corticosterone EIA, and one group-specific 5a-pregnane-3b,11b,21-triol-20-one EIA) in deermice by evaluating FCM changes as a function of diurnal rhythm and sex, and by challenging them with dexamethasone, ACTH, predator calls, and temperature extremes. Although we acknowledge low sample sizes, our results validated only the two EIAs. We found that dexamethasone suppressed FCM levels within ~ 10 h post injection, whereas ACTH caused an increased trend in FCM levels within ~ 2 hours post injection. Only the group-specific EIA detected differences in FCM levels between sexes. Predator calls had no effect, but temperature extremes increased FCM levels. In the field, we performed two studies that evaluated the FCM response to the length of time in a live trap, using the EIAs. Both indicated that FCM levels increased with longer captivity times. Thus, we validated both EIAs in laboratory and field settings, and would recommend both for studying FCM relationships with disease dynamics in deermice.

# PHARMACOKINETICS AND PHARMACODYNAMICS OF ALFAXALONE AFTER A SINGLE INTRAMUSCULAR OR INTRAVASCULAR ADMINISTRATION IN MALLARD DUCKS (ANAS PLATYRHYNCHOS)

Tamara Kruse<sup>1</sup>, Mark Flint<sup>1</sup>, Kristen Messenger<sup>2</sup>, Andrew Bowman<sup>1</sup>, Turi Aarnes<sup>1</sup>, Thomas Wittum<sup>1</sup>

<sup>1</sup>The Ohio State University, <sup>2</sup>North Carolina State University

## Abstract

Developing safe and effective injectable anesthetic protocols in avian species can be challenging as many drug dosages cannot be predictably extrapolated from mammalian models. This study aimed to determine the pharmacokinetics and pharmacodynamics of alfaxalone in a 2-hydroxypropyl-beta-cyclodextrin formulation (Alfaxan<sup>®</sup>) in mallard ducks after single bolus injections of 10 mg/kg administered intramuscularly (IM) or intravenously (IV) (n=10), in a randomized cross-over design. A pilot study using 5 mg/kg administered IV was previously performed to assess safety on six ducks. Blood was collected at pre-determined times (0, 5, 10, 30, 60, and 90 minutes) following injection. Heart and respiratory rates and sedative effects were recorded for each time point to objectively assess plane of anesthesia. Blood samples were later analyzed by liquid chromatography/mass spectrometry (LC-MS) for plasma alfaxalone concentrations. Compartmental pharmacokinetics were used to analyze the plasma alfaxalone data. Mean (+ SD) Cmax following IM injection was 1.6 (+ 0.8) µg/ml, which occurred at 15.0 (+ 10.5) minutes. The mean plasma clearance of alfaxalone at 10 mg/ kg IV was 139.5 (+ 67.9) ml/min/kg. The elimination half-lives (mean [+ SD]) were 15.0 and 16.1 (+ 3.0) minutes following 10 mg/kg IV and 10 mg/kg IM, respectively. Mean bioavailability of alfaxalone at 10 mg/kg IM was 108.6%. The duration of nonresponsive sedation after each dose was approximately 6.0 (+ 2.0) minutes; however, none of the ducks reached an anesthetic plane that would allow for invasive procedures, such as surgery, to be performed. Heart and respiratory rates measured after administration of alfaxalone remained stable (HR range 148 to 180 bpm; RR range 17 to 21 bpm). All ducks recovered from alfaxalone sedation but were hyperexcitable during recovery. Based on sedation levels and duration, alfaxalone administered at dosages of 10 mg/kg in mallard ducks does not induce sustained anesthesia.

## WILD FELIDS MAY BE MORE SUSCEPTIBLE TO FELINE LEUKEMIA VIRUS (FELV) INFECTION DUE TO A LACK OF ENDOGENOUS FELINE LEUKEMIA

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### Abstract

Threats to wild felids include habitat destruction, depredation conflicts, and disease emergence. Incidence of pathogenic feline leukemia virus has increased in a number of wild felid species. The purpose of this studies is to examine the root of a contemporary FeLV outbreak in Florida panthers (Puma concolor coryi). Our phylogenetic analysis of the contemporary FeLV outbreak has further implicated domestic cats (Felis catus) as the origin of FeLV infections in wild felids. Furthermore, we detected a recombinant oncogenic variant in Florida panthers that is believed to be non-horizontally transmissible. Field studies have prompted us to examine the cellular basis of infection and intrinsic resistance to the virus. We hypothesize that wild felids may be at greater risk of FeLV infections of puma (P. concolor) and domestic cat cells, we have demonstrated that puma cells support greater infection and replication. Additionally, we documented related enFeLV elements in domestic cats that are relatively resistant to FeLV infection. Data-mining the domestic cat transcriptome showed that cells that are relatively resistant to FeLV transcribe more enFeLV elements than relatively susceptible cells. Wild felids lack enFeLV altogether, which may leave them more vulnerable to FeLV infection. As urbanization forces niche overlap and contact between wild and domestic cats in wild felids may continue to see increasing pressure from FeLV infection. It is imperative to consider domestic cats in wild felid conservation action plans.

# WILD BIRDS PRESENCE, MOVEMENTS AND INTERACTIONS WITH HOUBARA BUSTARDS ASSESSED IN CONSERVATION BREEDING SITES IN THE UNITED ARAB EMIRATES

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### Abstract

Breeding facilities are known to attract wildlife, especially birds, as a source of food and water. This is notably obvious in arid environment, where they act as oasis in the middle of desert. In such situation, interactions between wildlife and livestock are of concern because of potential pathogens spillover.

This study aimed to combine different methods to describe and quantify the risk represented by wild birds for captive populations, by using the example of conservation breeding of Asian Houbara bustard (Chlamydotis macqueenii) in the United Arab Emirates. Three approaches were used: (i) inventory of bird species occurring on breeding sites and their surroundings, (ii) description of movements of main resident species in and out the breeding sites and potential exchanges with surrounding sites, (iii) assessment of nature and frequency of contacts between wild birds and captive bustards. These data are key for better understanding of the potential for pathogens transmission between wild and captive populations and formulate recommendations for sanitary control strategies.

During two consecutive years, 4782 birds of 56 species were captured and marked on two breeding sites and 5162 birds of 45 species were spotted in their surroundings. Focusing on the dominant resident species, subcutaneous transponders and VHF transmitters were used to track external movements from and toward breeding sites. Finally, bird counts in aviaries areas (2053 birds from 21 species) allowed describing wild birds interactions with captive bustards.

This study, using multimodal approach in poorly studied ecosystems, provides original results. They demonstrates that wild bird communities of breeding sites and their surroundings are slightly different, but both are dominated by few resident species. Moreover, birds from breeding sites are moving out frequently during round trips to surrounding sites. However, only a small fraction of them is interacting with captive bustards.

# REVEALING VIRUS DIVERSITY IN URBAN WILD BIRDS WITH NEUROLOGICAL SYMPTOMS USING META-TRANSCRIPTOMICS

Wei-Shan Chang<sup>1</sup>, John-Sebastian Eden<sup>2</sup>, Karrie Rose<sup>3</sup>, Mang Shi<sup>4</sup>, Edward Holmes<sup>4</sup>

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## Abstract

**Background and objective**: Wild birds are major natural reservoirs and potential dispersers of a variety of infectious diseases. It is therefore of considerable importance to understand their viral diversity for both conservation and to determine the risks for spill-overs to humans and other wildlife. We investigated the potential infectious causes in native avian species in Australia presenting with neurological symptoms and suspected underlying virus pathogens.

**Materials and Methods**: RNA from diseased birds were extracted and pooled based on tissue types and clinical manifestation for library preparation and sequencing. By using a bulk meta-transcriptomic approach (i.e. unbiased RNA-sequencing), we identified a number of novel viruses from the families Astroviridae, Picornaviridae, Polyomaviridae, Paramyxoviridae, Parvoviridae, Flaviviridae, and Circoviridae in common urban wild birds including magpies, magpie-park, pied currawongs, ravens, and rainbow lorikeets. The underlying aetiology of each case was then confirmed by PCR assays targeting the novel pathogens identified.

**Results and conclusions**: Our results revealed a number of candidate viral pathogens possibly contributing to neurological pathology in black and white bird diseases and clenched claw syndrome in Australia wild birds. Importantly, the existence of a diverse virome in urban avian species highlights the importance of elucidating the ecology of wildlife pathogens in urban environments, which will become increasingly important for managing disease risks and surveillance indicators to wildlife and humans. More broadly, our work shows how meta-transcriptomics has revolutionized pathogen discovery in wildlife diseases.

# OUTBREAK OF AVIAN PARAMYXOVIRUS 1 (APMV-1) IN JUVENILE DOUBLE-CRESTED CORMORANTS (PHALOCROCORAX AURITUS) IN NEW YORK STATE: DIAGNOSED BY HISTOPATHOLOGY BUT MISSED BY MATRIX PROTEIN RRT-PCR TESTING

Carmen Smith, Edward Dubovi, Randall Renshaw, Laura Goodman, Amy Glaser, Cynthia Hopf, Sara Childs-Sanford, María Forzán

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## Abstract

Thirteen juvenile double-crested cormorants (Phalacrocorax auritus) were found dead or moribund across New York State, USA, from August to October 2018. All moribund birds showed signs of neurologic dysfunction, such as wing drooping, dullness, and lethargy. On necropsy, most of the birds (9/13, 69%) were emaciated, 2/13 (15%) had sciatic nerve enlargement and 2/13 (15%) had hemorrhagic enteritis. Histologically, 7/9 (78%) birds for which the central nervous system (CNS) was in sufficiently good condition for examination had encephalitis characterized by lymphocytic perivascular cuffing in the cerebrum, cerebellum, brainstem, or optic nerve. Microscopic examination of CNS from the remaining four cormorants was inconclusive due to advanced autolysis. Suspecting avian paramyxovirus type 1 (APMV-1) infection, samples (brain, lung, kidney) from these birds were tested using the National Veterinary Services Laboratories (NVSL) Newcastle disease rRT-PCR assay. This assay, with primers for the highly conserved viral matrix protein, is thought to amplify most genotypes of APMV-1 across avian species despite being designed to detect Newcastle disease in poultry. Five cormorants tested positive for the matrix protein but negative for highly virulent strains. Subsequent virus isolation along with PCR and sequence analysis of part of the fusion protein gene from 6 birds that had tested negative or suspect for the matrix protein (7/13, 46%) or positive for matrix protein (6/13, 54%) but negative for highly virulent strains (6/6, 100%) indicated a cleavage site sequence (KRQKRF) consistent with highly virulent strains of AMPV-1. Our findings support what has previously been reported, that the standard NVSL assay used to identify highly pathogenic paramyxovirus in poultry targeting the fusion protein is inadequate to detect highly pathogenic APMV-1 strains in cormorants, and highlight the importance of development and validation of tests specific to pathogens affecting wildlife species.

#### AVIAN INFLUENZA VIRUS DETECTION VIA REMOTE SENSING AND SIZE EXCLUSION CHROMATOGRAPHY

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<sup>1</sup>University of California Davis, <sup>2</sup>UC Davis Population Health & Reproduction

### Abstract

Migratory waterfowl such as geese and ducks are thought to be the primary reservoirs of Avian Influenza, and thus are implicated as a mechanism by which AI is spread to commercial poultry. USDA led surveillance efforts of waterfowl for AI have been discontinued in the contiguous United States. Therefore, prediction of waterfowl location and density is highly advantageous to direct what limited AI surveillance resources are available. UC Davis and University of Delaware have developed a web application that utilizes various remote sensing technologies to identify and/or predict waterfowl densities in the Central Valley of California. Utilizing this web-tool, we identified wetlands with high waterfowl density for water sampling via size exclusion chromatography coupled to multi-segment PCR for AI virus detection. Our preliminary data demonstrate that size exclusion chromatography is an effective method for the concentration of AI viruses followed by multi-segmented PCR and sequencing. This detection method offers a novel, sensitive and targeted approach for AI surveillance in order to better understand the dispersal of AI into the environment from the primary reservoir.

### HISTOPATHOLOGY ASSOCIATED WITH DEMODEX SP. INFESTATION IN SOUTHERN SEA OTTERS (ENHYDRA

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## Abstract

Mites of the genus Demodex (Acari: Demodicidae) inhabit the skin of virtually all mammals. In this study, we describe parasitic Demodex sp. mites from the integument of southern sea otters (*Enhydra lutris nereis*). In some cases, clinically significant Demodex sp. infestation such as demodicosis may be associated with inflammation, follicular rupture, and alopecia. Due to their aquatic habitat and heavy reliance on water-repellent fur for insulation, the development of demodicosis could be harmful or fatal for sea otters, a federally-listed threatened species. We examined formalin-fixed, paraffin-embedded integument from the head, genitals, flipper, paws, and other alopecic areas from 20 deceased otters for Demodex sp. mites and any evidence of mite-associated histopathology. Intrafollicular mites were observed in the integument of 11 otters (55%), 5 of which (54.5%) had pelage changes characteristic of demodicosis in other mammalian hosts. In sea otters, microscopic visualization of intrafollicular Demodex sp. mites was spatially-associated with dermal pigmentary incontinence, plugging of follicular ostia with admixed sebum and keratin, and lymphoplasmacytic perifollicular and perivascular inflammation. Histological confirmation of demodicosis in southern sea otters may have important implications for clinical care, conservation, and rehabilitation.

## USING RAT RELATEDNESS TO UNDERSTAND HETEROGENEOUS PATHOGEN PREVALENCE IN AN URBAN ENVIRONMENT

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## Abstract

Urban rats are carriers of several zoonotic pathogens, and the prevalence of these pathogens can vary across fine spatial scales (i.e., by city block). These patterns may be driven by rat ecology, such that minimal movement of individuals among city blocks restricts pathogen spread. The objective of this study was to evaluate the population genetic structure and movement patterns of rats across an urban landscape, and to understand how these dynamics relate to the distribution of a pathogenic bacterium (*Leptospira interrogans*) that they carry.

We trapped 615 Norway rats (*Rattus norvegicus*) in 32 city blocks in Vancouver, Canada. We collected liver samples for genetic analysis and kidney samples to test for L. interrogans carriage. We identified over 15,000 single nucleotide polymorphisms and used them to classify individuals into genetically similar clusters using Discriminant Analysis of Principal Components. We also used pedigree analysis to identify parent-offspring and sibling relationships among sampled individuals. Using inferred movement events, where closely related individuals were trapped in different city blocks, we described areas where migration rates were lower or higher than expected. Finally, we compared patterns of relatedness and movement to prevalence data for L. interrogans.

We identified 11 genetic clusters of rats with strong variation in the degree of spatial structuring. Where rat densities were high, one genetic cluster dominated a city block, while low-density areas showed more evidence of movement. Relatedness was high among pairs of rats trapped within 50m of one another and decreased past 250m. Prevalence of L. interrogans varied by block (0 – 66%). Pedigree analysis and comparisons to disease prevalence is ongoing.

Our results suggest that spatial clustering of related individuals could support heterogeneous patterns of pathogen prevalence, and that risk of pathogen spread among city blocks may be higher in low-density areas where rat movement was greater.

# A RETROSPECTIVE SUMMARY OF CERVID MORTALITY IN ONTARIO AND NUNAVUT REGIONS OF CANADA (1991-2017)

Samantha E. Allen, Nadine A. Vogt, Claire Jardine, Nicole Nemeth

University of Guelph

### Abstract

The causes of mortality in free-ranging cervids range from anthropogenic to environmental conditions that may affect habitat suitability, food availability and predator dynamics. Wildlife species in Ontario/Nunavut, Canada, are experiencing challenges from climate change, human population expansion and northward expansion of vector-borne pathogens.

We retrospectively evaluated passive diagnostic data from wild cervids submitted to the Ontario/Nunavut node of the Canadian Wildlife Health Cooperative (CWHC) from 1991 to 2017 (n=584). Submissions encompassed 4 species, most commonly the white-tailed deer (Odocoileus virginianus) (n=211), followed by moose (Alces alces) (n=140), elk (Cervus canadensis) (n=138) and caribou (Rangifer tarandus) (n=95). Most submissions involved postmortem examination (gross and histopathology) of carcasses, but in some cases, field-collected tissues were submitted. Ancillary laboratory tests were performed on a case by case basis. Univariable logistic regression was used to examine the association between select common causes of mortality and the following: age and season. Infectious causes accounted for the majority of deaths among all species (n=199), most commonly bacterial (n=91). In the non-infectious (n=197) group, trauma (n=122) was the most commonly diagnosed cause of mortality, followed by emaciation (n=48).

The odds of a cervid being diagnosed with an infectious cause of mortality were significantly lower in juveniles compared to adults (p<0.001). Trauma was most commonly attributed to collision (n=40) with moving objects, and there was no significant association between the odds of a trauma-related mortality and age (p=0.18). The odds of a diagnosis of emaciation were significantly higher in juveniles compared to adults (p<0.001). Compared to the fall, the odds of emaciation-related deaths were significantly higher in the spring (p<0.001) and winter (p<0.001).

These results reveal that anthropogenic and environmentally-associated deaths are commonly diagnosed in cervids submitted to the CWHC in the Ontario/Nunavut region, and can be used in the development of future population management strategies.

## MOLECULAR CHARACTERIZATION AND PREVALENCE OF HALARACHNE HALICHOERI IN THREATENED SOUTHERN SEA OTTERS (ENHYDRA LUTRIS NEREIS)

Risa Pesapene<sup>1</sup>, Erin M. Dodd<sup>2</sup>, Nadia Javeed<sup>2</sup>, Melissa Miller<sup>2</sup>, Janet Foley<sup>1</sup>

<sup>1</sup>UC Davis, <sup>2</sup>California Department of Fish and Wildlife

### Abstract

Parasitism, particularly in concert with other sublethal stressors, may play an important, yet underappreciated role in morbidity and mortality of threatened species. During necropsy of southern sea otters (*Enhydra lutra nereis*) from California submitted to the Marine Wildlife Veterinary Care and Research Center's Sea Otter Necropsy Program between 1999 and 2017, pathologists occasionally observed nasopulmonary mites infesting the respiratory tracts. Infestation was sometimes accompanied by lesions reflective of mite-associated host tissue damage and respiratory illness. Our objectives were to estimate prevalence of nasopulmonary mites, determine the taxonomic identity of the observed mites, and create a DNA reference for these organisms in southern sea otters as an aid in population management. Using unique morphological characteristics discerned via light and scanning electron microscopy (SEM), we identified the mites as *Halarachne halichoeri*, a species typically associated with harbor seals (*Phoca vitiluna*). The 18S, 16S, 28S and ITS1-2 genetic regions were sequenced and submitted to GenBank. We observed *H. halichoeri* mites in 25.6% (95% CI 19.9–33.4%) of southern sea otters from a subset of necropsies performed between 2012 and 2017. This is the first documentation of *H. halichoeri* in southern sea otters and harbor seals.

### A POLYOMAVIRUS DETECTED IN AMERICAN BLACK BEAR (URSUS AMERICANUS)

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<sup>1</sup>University of California Davis, <sup>2</sup>Charles University

## Abstract

Polyomaviruses (PyVs) are a family of DNA tumor viruses that co-evolve with mammalian host animals[BC([1]. The number of members included in the Polyomaviridae family is growing rapidly, especially thanks to combination of viral metagenomics and degenerate primer strategies. Among the Carnivora order, PyVs have been identified in sea lions, badgers, Hawaiian monk seals, red pandas, Weddell seals, and dogs. A polyomavirus found in raccoons appears to cause neuroglial tumors. We hypothesized that PyVs were the cause of a series of nerve tissue tumors in black bear (Ursus americanus). Using degenerate primers for primer walking, we were able to sequence the whole genome of a novel polyomavirus, UaPyV. Viral sequence was obtained from three nervous system tumors but was not present in normal brain tissue from two bears. Phylogenetic analysis is consistent with co-evolution of UaPyV with ursids. It will be important to investigate the possible causal relationship between a new member of the Polyomaviridae family, UaPyV, and neoplasia.

# EVALUATION OF FLUMETHRIN-IMPREGNATED COLLARS FOR PREVENTION VOF SARCOPTIC MANGE IN AN URBAN POPULATION OF ENDANGERED SAN JOAQUIN KIT FOXES (VULPES MACROTIS MUTICA)

Jaime Rudd<sup>1</sup>, Brian Cypher<sup>2</sup>, Deana Clifford<sup>3</sup>, Janet Foley<sup>1</sup>

<sup>1</sup>University of California, Davis, <sup>2</sup>CSU Stanislaus, <sup>3</sup>California Department of Fish and Wildlife

### Abstract

Sarcoptic mange epidemics can have long-lasting impacts on susceptible wildlife populations, potentially contributing to localized population declines and extirpation. Since 2013, there have been 460 reported cases of sarcoptic mange in an urban population of endangered San Joaquin kit foxes (Vulpes macrotis mutica) in Bakersfield, California. As part of a multi-faceted, large-scale response to mitigate mange-caused mortalities and reduce this conservation threat, we conducted a 2-year randomized field trial to assess the ability of long-acting flumethrin collars to protect foxes from sarcoptic mange. We hypothesized that kit foxes wearing flumethrin collars would have lower risk of mange infestation compared to uncollared kit foxes. We captured, examined, and administered selamectin to 35 kit foxes living in a high-density population on the CSUB campus and randomly assigned each fox to either receive a flumethrin collar placed within a VHF radio collar or a VHF radio collar without flumethrin. The survival and mange-infestation status of study animals was monitored via radio telemetry, remote cameras and periodic recapture examinations and compared among treated and control kit foxes using a Cox PH model. The average time to onset of mange for treated kit foxes (176 days, N=17) was similar to controls (171 days, N=18). Although treatment with flumethrin did not significantly reduce the onset of mange in days for all kit foxes, when adjusting for mange at the time of study recruitment, there was a delay in mange development for treated kit foxes (174 days, N=10) compared to controls (83 days, N=5; p < 0.01). Sex was not a significant predictor of time till mange onset. This study demonstrates an approach to evaluating population-level protection and contributes to the limited literature on efficacy, safety, and practicality of acaricides in free-ranging wildlife.

# COLONIZATION PATTERNS OF CHRONIC HEMO-PARASITE INFECTIONS IN AFRICAN BUFFALO (SYNCERUS CAFFER): EVIDENCE FOR THE COMPETITION - COLONIZATION TRADE-OFF?

Caroline Glidden, Chenyang Duan, Yuan Jiang, Jan Medlock, Brianna Beechler, Anna Jolles

Oregon State University

#### Abstract

### Background/Question/Methods:

Preliminary studies suggest parasite communities follow patterns of succession synonymous to free-living systems, with colonization patterns contributing to long term parasite community stability. However, few studies have examined succession dynamics in long-lived mammals. Here, we sampled 40 African buffalo calves every two months for three years. We used high-throughput amplicon sequencing to quantify relative abundance of tick transmitted hemo-parasites within each sample. We then utilized novel statistical framework, that accounts for the compositional nature of our data as well as repeated measures study design, to model how presence and relative abundance of each parasite changes with age. We used our statistical models to calculate age of first infection and rank in developed communities for each parasite.

#### **Results/Conclusions**

We found evidence supporting the competition-colonization tradeoff theory as certain species were consistently the first species to infect calves (colonizers) but species that infected calves at least secondarily obtained highest relative abundances when communities reached equilibrium (competitors). Our findings suggest succession dynamics may contribute to co-existence of tick transmittedhemo-parasite species in African buffalo and could have important implications for transboundary disease management.

#### Implications

Interestingly, African buffalo and cattle can be infected with the same species of hemo-parasites. However, species that are colonizers in African buffalo are dominant species in cattle whereas species that are dominant in African buffalo are found at low prevalences in cattle. In southern Africa, cattle are often treated for ticks meaning that they might be constantly turned into new "habitat patches" for tick borne hemo-parasites (i.e. well suited for parasites that are better colonizers). Thus, future directions should explore if differences in hemo-parasite communities between African buffalo and cattle are a product of ecological scale disease management or evolutionary scale host specificity.

## PATHOGEN PREVALENCE AND GENE DIVERSITY IN AN ENDANGERED PASSERINE, THE FLORIDA GRASSHOPPER SPARROW (AMMODRAMUS SAVANNARUM FLORIDANUS)

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### Abstract

An important concern for conservation breeding programs is minimizing the risk of transfer of novel or high-risk pathogens between captive and wild populations. The Florida Grasshopper Sparrow (Ammodramus savannarum floridanus) is a critically endangered species with less than fifty breeding pairs left in the wild. A conservation breeding program has been established with the goal of supporting the recovery of the wild population. Unfortunately, birds in the captive breeding program initially have tested positive for pathogens, including Eastern Equine Encephalitis, Rickettsia, Ehrlichia, coccidians, haemosporidians, and microfilaria. The objectives of this study are to 1) determine if known pathogens are co-prevalent in wild and captive populations of the Florida Grasshopper Sparrow and to 2) test for associations between bird "fitness" and levels of genome-wide diversity. For objective 1, the goal was to make inferences about whether pathogens detected ex sit, are unique to captive birds. To test this hypothesis, we screened blood, fecal, and oral samples from both wild and captive bird populations for vector borne diseases and direct transmission pathogens using PCR with Sanger sequencing, Q-PCR, blood film review, and fecal culture. A novel coccidian and a novel microfilaria were identified in both populations. For objective 2, associations between pathogen prevalence were tested, as well as ecological and demographic parameters (e.g. nesting success, mate selection) with genome-wide diversity. Preliminary results reflect a ~6% infection rate across all 2017-18 wild birds (n = 66) tested for at least one assay. Sequencing of cytochrome b mitochondrial genes have shown that parasite species detected in both captive and wild populations are 98% genetically identical. We will present results on genome-wide heterozygosity correlations with pathogen prevalence and other fitness metrics for this endangered species. This study's results provided a comprehensive health profile for this species and led to the decision to release captive birds.

# TRYPANOSOMA CRUZI (AGENT OF CHAGAS DISEASE) AND SARCOCYSTIS SPP. IN ENDANGERED SOUTH TEXAS OCELOTS (LEOPARDUS PARDALIS)

Italo Zecca<sup>1</sup>, Carolyn Hodo<sup>1</sup>, Heather Prestridge<sup>1</sup>, Thomas deMaar<sup>2</sup>, Hilary Swarts<sup>3</sup>, Jessica Light<sup>1</sup>, Sarah Hamer<sup>1</sup>

<sup>1</sup>Texas A&M University, <sup>2</sup>Gladys Porter Zoo, <sup>3</sup>US Fish and Wildlife Service

### Abstract

The federally endangered ocelot (Leopardus pardalis) population of South Texas is declining due to habitat destruction and animal vehicle collisions, with less than 80 ocelots remaining. The impact of infectious diseases on ocelot health is largely unknown, owing in part to the challenges of sample collection from these elusive and protected cats. South Texas is an area with robust transmission of Trypanosoma cruzi, the protozoan parasite of Chagas disease, and our previous studies highlighted high prevalence among domestic dogs (>20%) and triatomine 'kissing bug' vectors (>55%) in the region. Further, we found that up to 11.4% of south Texas feral cats may be infected, with some having cardiac inflammation, yet the impact of this parasite on the health of sympatric wild cats is unknown. We received ocelot tissue from salvaged animals that were installed in collections (BRTC-TAMU and Gladys Porter Zoo); carcasses were collected by U.S. Fish and Wildlife Service following death primarily by vehicle collision from 2010-2017 around Laguna Atascosa National Wildlife Refuge in South Texas. From 21 individuals, we subjected skeletal muscle (n=15), heart tissue (n=5), blood clot (n=7), serum (n=1) and other organs (n=4) to protozoal parasite PCR. T. cruzi DNA was detected in samples of two different ocelots (9.5%) with infection found in skeletal muscle and blood clot; subsequent strain typing showed parasite discrete taxonomic unit 'Tcl'- the DTU previously implicated in human Chagas disease in the US. On histologic examination, protozoal schizonts or cysts with bradyzoites were seen in four animals (19%)- all were confirmed as Sarcocystis spp. using PCR. Advanced autolysis hindered the histologic examination of the T. cruzi positive muscle. Scientific collections are essential in facilitating endangered species infectious disease research. Coupled with live animal surveillance and analysis of sympatric felid species, we may advance the understanding of parasitic disease in ocelots.

# THE IMPACT OF SAMPLING INTERVAL ON SALMONELLA SEROTYPES AND SUSCEPTIBILITY PATTERNS OF SALMONELLA AND ESCHERICHIA COLI: A LONGITUDINAL STUDY OF RACCOONS (PROCYON LOTOR) IN SOUTHERN ONTARIO, CANADA (2011-2013)

Nadine A. Vogt<sup>1</sup>, David L. Pearl<sup>1</sup>, Nicol Janecko<sup>1</sup>, Richard J. Reid-Smith<sup>1</sup>, Kristin Bondo<sup>2</sup>, Samantha E. Allen<sup>1</sup>, Claire Jardine<sup>1</sup>

<sup>1</sup>University of Guelph, <sup>2</sup>University of Calgary

### Abstract

Wildlife species such as raccoons (Procyon lotor) are particularly well adapted to anthropogenic environments and may contribute to the transmission of bacteria potentially harmful to human and animal health.

We performed a longitudinal study of the carriage of Salmonella and Escherichia coli to determine how Salmonella serotypes and antimicrobial resistance in Salmonella and E. coli varied within an individual animal over time, and whether the sampling interval was associated with a change in serotype or susceptibility pattern. Fecal swabs were obtained from raccoons trapped monthly from June to November at 10 sites in southern Ontario between 2011 and 2013. Swabs were tested for Salmonella and non-specific E. coli, and susceptibility to 15 antimicrobials was determined using a modified NARMS panel. Among raccoons sampled on at least two separate occasions, we identified subsets of raccoons with a) at least two Salmonella isolates; b) at least two E. coli isolates. Using multilevel logistic regression with a random intercept for animal, we examined whether the sampling interval was associated with the following outcomes: 1) change in susceptibility pattern; 2) change in Salmonella serotype.

Multiple E. coli isolates were obtained from 258 animals, and multiple Salmonella isolates were obtained from 44 animals. Raccoons were trapped between 2 and 8 times, and the sampling interval ranged from 25 to 819 days. Sampling interval was not significantly associated with any outcome (p>0.05), but the random effect for animal was significant.

Our inability to detect an association between sampling interval and a change in serotype or susceptibility may be a true finding or related to study design limitations, and future work may benefit from assessing a shorter sampling interval and collection of multiple isolates per animal. Finally, detection of autocorrelation suggests that stability or variation in serotypes and susceptibility patterns is raccoon-dependent.

# INITIATING SURVEILLANCE FOR THE PATHOGENIC FUNGUS, BATRACHOCHYTRIUM SALAMANDRIVORANS, IN HIGHLAND COSTA RICAN SALAMANDER POPULATIONS

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### Abstract

For roughly five decades, emergent infectious diseases have directly contributed to catastrophic declines in global amphibian populations. Now, with 18% of species listed as endangered or critically endangered, amphibians are the most endangered group of vertebrates. Since 2008, the emergent fungal pathogen, Batrachochytrium salamandrivorans (Bsal), has caused significant population declines in European salamander species, sparking international concern. The emergence of this pathogen has been met with significant proactive political action, surveillance work, and research efforts in Europe, the United States, Canada and Mexico. No such work has been conducted in Costa Rica, a country: 1) rich in salamander species diversity, 2) mountainous habitat suitable for the persistence and increased pathogenicity of Bsal, 3) high likelihood for pathogen pollution from tourist visitation, 4) declining salamander abundance. With a multibillion-dollar ecotourism industry, (~14% from Europe, where Bsal has become a concern), we believe Costa Rica's salamander community to be at significant risk of Bsal introduction, meriting proactive surveillance and research efforts. Our objective was to initiate surveillance efforts for Bsal within highland salamander populations in the Central and Talamanca Mountains of Costa Rica, areas that would be very ecologically suitable for Bsal persistence. We hypothesized that if we found evidence of Bsal it would in regions with heavy tourism. Between November and December of 2018, 42 salamanders representing 3 species (Bolitoglossa subpalamata, B. pesrubra, and Nototriton abscondens) were sampled across multiple sites, including one of Costa Rica's most visited national parks. Bsal was not detected. Samples were analyzed using PCR at the Southeast Cooperative Wildlife Disease Study at the University of Georgia. Further surveillance efforts are ongoing. Surveillance results were a specific requirement by the Costa Rican natural resource agency (CONAGEBIO) prior to obtaining a permit to conduct susceptibility trials.

# PCR SURVEILLANCE FOR LEPTOSPIRA INTERROGANS IN ALASKAN CARIBOU (RANGIFER TARANDUS GRANTI) AND REINDEER (RANGIFER TARANDUS TARANDUS)

Melissa Clark, Cristina Hansen

University of Alaska Fairbanks

### Abstract

Leptospirosis is a zoonotic bacterial disease that is transferred between mammals through the contamination of urine, carcasses, and waterways. This disease is caused by Leptospira interrogans, which is spiral-like in nature and thrives in moist environments. There are currently over 200 described serovars of Leptospirosis worldwide. In ungulates, Leptospirosis has been known to cause subclinical to fatal clinical signs; including fever, anorexia, renal failure, and mortality. Of these known serovars, six are known to circulate in North American ungulates. There is little understanding of the status of Leptospirosis in Alaskan Rangifer species. Our objectives are to determine the prevalence of leptospirosis in Alaskan Rangifer (caribou and reindeer) species through the surveillance of blood and urine. We performed PCR to amplify the 16S rRNA gene of Leptospira interrogans in blood and urine samples and visualized PCR product using agarose gel electrophoresis. Zero of 84 blood samples were positive for Leptospirosis; that leptospiral organisms remain in the kidneys, and is periodically shed out in the urine. These positive samples have displayed negative titres for Leptospirosis, indicating a possible new, or undetected serotype in herds.

## HEPATOCYSTIS MALARIAL INFECTION AND ANTIVIRAL IMMUNE GENE EXPRESSION IN AFRICAN FRUIT BATS - IMPORTANT ZOONOTIC RESERVOIRS

Jordan Simpson<sup>1</sup>, DeeAnn Reeder<sup>1</sup>, Kenneth Field<sup>1</sup>, Imran Ejotre<sup>2</sup>, Juliane Schaer<sup>2</sup>

<sup>1</sup>Bucknell University, <sup>2</sup>Humboldt University

### Abstract

Bats are unique both ecologically and physiologically, and can thrive in challenging seasonal environments. Despite their role in zoonoses, bat immunology and disease ecology are relatively poorly understood. We have demonstrated that the highly social and peri-domestic African fruit bat Epomorphorus labiatus, which may play a role in Filovirus disease ecology, hosts high levels of the malarial parasite Hepatocystis year-round. Our data show that infection load (percent RBCs infected) is significantly correlated to electrolyte imbalance, thus potentially compromising the bat's overall health status. We will explore the hypothesis that being constitutively infected with one pathogen (Hepatocystis) is correlated with changes in antiviral immunity and overall bat health. For this study, Hepatocystis infection levels were quantified by scanning blood smears and by measuring levels of parasite transcripts by 3'-TagSeq next generation sequencing transcriptomic gene expression analysis in liver tissue from 100 little epauletted fruit bats. Host and parasite gene expression was measured by mapping the 3'-TagSeq data against a high quality de novo transcriptome assembly generated from deep-sequencing RNA-Seq of spleen and liver. Weighted correlation network analysis (WGCNA) will be used to explore relationships between the level of parasite infection with host gene expression profiles during bat life history stages. Host-parasite interactions will be characterized by identifying clusters (pathways) of highly correlated genes. Demonstration of an altered immune gene expression profile in those bats with higher Hepatocystis prevalence coupled with impacted bat health may provide insight into how these fruit bats are successful viral reservoir hosts.

#### USING AGENT-BASED MODELING TO IMPROVE OUR UNDERSTANDING OF CHRONIC WASTING DISEASE

Noelle Thompson, Sonja Christensen, David Williams, William Porter

Michigan State University

### Abstract

Chronic wasting disease (CWD) continues to grow in regions where the disease is endemic and emerge in formally unaffected areas. Despite efforts to control CWD, uncertainties about the ecology of this disease continue to limit our understanding of it and our ability to find management solutions. Agent-based modeling (ABM) provides a modeling framework that allows us to simulate the complexity inherent to wildlife disease systems. ABM uses a bottom-up approach where the population and environment being simulated are defined by the variation of individuals it comprises. In effect, it captures two properties that are essential for projecting infectious disease outbreaks but that are often missed by traditional models: emergent behaviors and interactions between individuals and the environment. Our objective was to demonstrate the ability of ABM to manage the complexities of CWD. We developed a stochastic, spatially explicit epidemiological model using an agent-based framework as an approach to address past limitations and uncertainties and, thus, improve model applicability. We used Michigan field data and disease data from endemic areas of the United States to construct this model. We developed two interrelated submodels within our ABM: a free-ranging white-tailed deer (Odocoileus virginianus) population model and a CWD model. We discuss the limits associated with modeling CWD and our means of addressing them within this model. We discuss the implications of our model for 1) contributing to the knowledge of CWD transmission in free-ranging deer populations and 2) improving the ability of wildlife agencies to control the disease through the identification of management strategies that can reduce prevalence and spread of CWD in free-ranging deer populations. This paper illustrates the ability of ABM to address the complex nature of CWD and its interactions with the environment.

## RE-EMERGENCE OF LEPTOSPIROSIS IN CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS) AFTER A FOUR-YEAR HIATUS: DEMOGRAPHICS, SURVIVAL, AND CLINICAL FEATURES OF THE 2017-2018 EPIZOOTIC

Emily Whitmer<sup>1</sup>, Pádraig Duignan<sup>1</sup>, Frances Gulland<sup>2</sup>, Shawn Johnson<sup>1</sup>, Abby McClain<sup>3</sup>, Carlos Rios<sup>1</sup>, Dane Whitaker<sup>1</sup>, Sophie Whoriskey<sup>1</sup>, James Lloyd-Smith<sup>4</sup>, Katherine Prager<sup>4</sup>, Cara Field<sup>1</sup>

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### Abstract

The spirochete bacterium Leptospira interrogans serovar Pomona is an enzootic pathogen in California sea lions (Zalophus californianus; CSL). Clinical cases have been admitted to California rehabilitation centers in most years since the 1980s with larger seasonal epizootics on a three- to five-year cycle. Based on surveillance of wild-caught animals and data from stranded animals, pathogen circulation in the population appeared to have essentially ceased from 2013 until mid-2017. The pathogen re-emerged in 2017, with 356 cases admitted to The Marine Mammal Center in Sausalito, CA between July 2017 and December 2018. The greatest geographic concentration of clinical cases was along California's central coast, with 51% of strandings in Monterey and Santa Cruz counties. Sea lion demographics and proportion of cases surviving to release were similar to previous epizootics. Males represented 92% of all cases and juveniles represented 56%. Survival to release was 42%, and survival of juveniles was higher than that of other age classes (P<0.001) but survival of each sex was similar. Mean serum blood urea nitrogen, creatinine, phosphorus, sodium, total bilirubin, and gamma-glutamyl transferase were significantly lower on admission in animals that survived to release as compared to those that did not (P<0.02). Mean potassium and total calcium were significantly higher on admission in animals that survived to release (P<0.001). Odds of survival to release was 102 times greater for animals that ate voluntarily within six days of admission (95% CI 23.8-474.3, P<0.0001). Based on these provisional results, we propose a set of prognostic indicators based on serum chemistry and clinical signs that can be utilized for triage of CSLs with leptospirosis.

# ASSESSING INFLUENZA A VIRUS INFECTION AND EXPOSURE IN A BREEDING SEABIRD COLONY ON MIDDLETON ISLAND, ALASKA

Brianna Williams<sup>1</sup>, Andrew Ramey<sup>2</sup>, Scott Hatch,<sup>3</sup> David Stallknecht<sup>4</sup>, Michael Yabsley<sup>1</sup>

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### Abstract

The role of wild birds in the circulation of influenza A virus (IAV) has been widely studied. However recent evidence suggests the ecological role of seabirds (Charadriiformes and Phalacrocoracidae) in the transmission of IAV may differ from the more widely studied waterfowl (Anseriformes). To gain further inference regarding possible role of seabirds in IAV ecology in Alaska, we sampled six species of seabirds (Rissa tridactyla, Phalacrocorax pelagicus, Cerorhinca monocerata, Uria aalge, Fratercula cirrhata, and Larus glaucescens) breeding on Middleton Island during June- August 2014 and 2015. For IAV detection, cloacal swabs and environmental fecal samples were tested by virus isolation and/or RT-PCR. To detect IAV antibodies, serum samples were tested with a commercially available bELISA (IDEXX Laboratories, Inc.). No IAV was detected in swab samples from 247 and 128 individuals or the environment sampled in 2014 and 2015, respectively. However, we detected a high prevalence of IAV antibodies in R. tridactyla, [106/219 (48.4%)] and U. aalge adults [90/107 (84.11%)] and lower prevalence in R. tridactyla [3/92 (3.3%)] and L. glaucescens [18/49 (36.7%)] chicks, C. monocerata [43/112 (38.4%)] and F. cirrhata [2/20 (20%)] adults, and P. pelagicus chicks [10/72 (13.9%)] and adults [15/62 (24.2%)]. There was no evidence of AIV antibodies in C. monocerata and F. cirrhata chicks. These data indicate that IAV exposure occurs in several of these understudied pelagic species of seabirds and that exposure probability may increase with age. Furthermore, the detection of IAV antibodies in unflighted chicks of three species provides evidence that IAV transmission may occur at this site during the breeding season even though no viruses were detected via virus isolation and RT-PCR.

#### EFFECTS OF SNAKE FUNGAL DISEASE ON EPIDERMAL MICROBIOME COMMUNITY DYNAMICS

Alexander Romer, Donald Walker

Middle Tennessee State University

### Abstract

Snake fungal disease (SFD), caused by Ophidiomyces ophiodiicola (O.o.), is an emerging infectious disease which affects free-roaming snakes in the Eastern United States. SFD is correlated with changes in the structure of epidermal bacterial and fungal communities in wild snakes. Snakes infected with SFD also display an increased shedding frequency, s0which is suspected to eliminate the pathogen from the epidermis. Both, disease state (SFD) and host-mediated factors (shedding) are associated with modifications to animal microbiomes. SFD and shedding can therefore be conceptualized as perturbations to the host microbiome, as described by disturbance theory, given that microbial communities are known to be altered by ecological disturbance. This work will elucidate the impact of shedding and SFD on the structure and function of the host microbiome. Differences in the effects of these processes on the microbiome are anticipated given the acute nature of shedding and the chronic nature of disease state. Common Gartersnakes (Thamnophis sirtalis) will be experimentally inoculated with O.o. to study the effect of disease state on the microbiome. The microbiome of all snakes will be sampled during a pre-shed state, during shedding, and then multiple times after shedding to document the response of the microbiome to the shedding process. High throughput sequencing and metagenomics will be used to characterize variation in microbiome community structure and function. Microbial abundance is expected to be significantly reduced on newly exposed epidermis immediately after shedding in all snakes. Microbiome function is expected to shift in infected snakes due to chemical and physical modifications to the epidermis resulting from disease state. Measuring these taxonomic and functional changes in the snake microbiome resulting from host and pathogen mediated disturbance will improve our understanding of host-microbiome-pathogen interactions and advance disturbance ecological theory in microbial community ecology.

#### SPATIAL CLUSTERING OF BAYLISASCARIS PROCYONIS INFECTION IN ONTARIO RACCOONS

Shannon French, David L. Pearl, Andrew Peregrine, Claire Jardine

University of Guelph

### Abstract

Baylisascaris procyonis, the raccoon roundworm, is distributed across North America and is an emerging zoonotic parasite. Reports of neurological disease associated with infection with the larval stage of this nematode have been reported in more than 150 species of birds and mammals. Our objective was to determine whether B. procyonis infections in raccoons cluster in space. Using spatial scan statistics with a Bernoulli model, we assessed 1353 Ontario raccoons submitted to the Canadian Wildlife Health Cooperative between 2013 and 2016 with known infection status. We identified a significant spatial cluster of increased infection prevalence in southern Ontario centred over the greater Toronto area (p <0.001) as well as a significant spatial cluster of decreased infection prevalence over southwestern Ontario (p <0.001). To examine for the potential influence of age and sex on the distribution, we repeated the scan with our data stratified first by age, then by both age and sex; the same spatial clusters were observed, suggesting that our unadjusted results were not confounded by age or sex. The recognition of these infection clusters will help guide future investigations that aim to establish the underlying geographic and anthropogenic factors associated with the occurrence of B. procyonis infection in raccoons. Furthermore, identification of a cluster of high infection prevalence in a major metropolitan area has implications for public education and risk management strategies due to the zoonotic potential of this parasite.

# BRIDGING THE GAP BETWEEN SCIENCE AND PRACTICE: PROTOCOLS TO FACILITATE BEST PRACTICES FOR ECOLOGICAL NICHE MODELING TO MAP DISEASE RISK

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### Abstract

Increasing concern for zoonotic disease emergence highlights the importance of identifying high-risk areas for spillover to human populations. Estimating the potential geographic distribution of these pathogens, as well as their hosts and reservoirs, could assist epidemiologists, public health practitioners and wildlife managers in their decision-making processes. Ecological niche modeling (ENM) has proven useful to predict the geographic distributions of many ecologically diverse organisms and is rapidly becoming common practice for disease risk mapping. Nevertheless, because complex biotic interactions often lie at the core of zoonotic disease systems, traditional conceptual and methodological frameworks may yield biologically unrealistic predictions, potentially leading to inefficient management strategies with dire socioeconomic consequences. We describe a new conceptual framework where appropriate selection between "black-box" and component-based modeling strategies depends on data availability (e.g. human outbreak data, parasite occurrence data) and the ecological idiosyncrasies (e.g. transmission mode, parasite life cycles) of each disease system. Additionally, this framework sets forth the concept of a micro-scale parasitic niche defined by host traits, aiming to aid in the identification of relevant biotic interactions for integration into ENMs. Once a realistic conceptual model is developed and the modeling strategy is defined, it should be possible to reliably forecast disease distributions via ENM. Computational tools for practitioners to map disease risk employing ENM currently exist; however, these often lack flexibility and transparency or require additional knowledge of a programming language, hindering their adoption. Wallace, a new modular R-based open-source software that implements ENM, lowers the entry barriers and fills this gap. With rich documentation in the GUI, Wallace guides users from acquiring and processing data through building models and examining predictions. Together, this proposed framework for disease ENM and new flexible software, like Wallace, can greatly improve disease risk mapping, enabling practitioners to make better informed decisions based on more reliable outputs.

## BAYLISASCARIS LARVAL MIGRANS IN WILD RODENTS AND LAGOMORPHS IN ONTARIO: A 30-YEAR RETROSPECTIVE ANALYSIS

Shannon French, David L. Pearl, Andrew Peregrine, Claire Jardine

University of Guelph

#### Abstract

The larval stage of the raccoon roundworm, Baylisascaris procyonis, is known to infect a wide variety of birds and mammals. Following ingestion of infective eggs from the environment, these accidental hosts can develop severe, potentially fatal, neurological disease. We investigated the incidence of lesions consistent with Baylisascaris sp. larval migration in lagomorphs and rodents submitted to the Ontario node of the Canadian Wildlife Heath Cooperative (CWHC) over a 30-year period. Examining the case reports for 879 animals submitted from 1988-2018, we identified animals with histological lesions typical of neural larva migrans (NLM) including the presence of inflammation and malacia of brain tissue, and in some cases, characteristic nematode larvae. Thirteen species of rodents and three species of lagomorph were submitted to the CWHC. Lesions consistent with Baylisascaris NLM were observed in 16% of submissions, representing 41% of those whose primary diagnosis was infectious in nature. Neural larval migrans were observed in 66% of submitted groundhogs (Marmota monax) which was the highest prevalence of lesions among examined species. Although Peromyscus sp. mice are believed to be the primary paratenic host for B. procyonis, only 10 animals were included in our dataset, none of which were positive for NLM. Therefore, the CWHC database may be better suited for surveillance of some species over others, resulting in potential gaps in knowledge overall. Nevertheless, determining the commonly affected species in Ontario will not only assist wildlife veterinarians, pathologists, and rehabilitation centers triage and diagnose individuals effectively, but also guide future work in identifying species important to the ecology of Baylisascaris sp. in Ontario.

### MICROCYSTIN-LR TOXICITY IN MALLARD DUCKS

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### Abstract

Clinical and pathological manifestations of exposure of aquatic birds to the hepatotoxic microcystin (MC) are poorly understood, and no formal case definition exists. We investigated single-dose oral toxicity of MC-LR in mallard ducks (Anas platyrhynchos) for 14 days. No mortality was observed. Low-dose oral MC-LR exposure was associated with recumbency and decreased ambulation, and all treated birds exhibited 0.8% to 11% weight loss. In the highest MC-LR dose (17.5 mg/kg), several blood hematology and biochemistry parameters changed drastically within 72 hours, but returned to normal within one week. In contrast, the lower MC-LR doses (1.75 and 5.5 mg/kg) exhibited a later and lower change in blood hematological and biochemical values. Hepatic and renal immunohistochemistry using antibodies directed against MC-LR on formalin-fixed, paraffin-embedded tissues revealed MC-LR labelling was strongest in bile canaliculi, and the apical region of renal proximal tubule epithelium. Histopathology and periodic acid-Schiff staining revealed discontinuity and suspected structural alterations of the basement membrane of renal proximal tubules and testicular seminiferous tubules, suggestive of nephrotoxicity and reproductive toxicity. Total microcystins (free and tissue-bound MC) were detected via gas chromatography in liver, kidney and testicle, but were not detectable in pectoral muscle or growing feathers with our analytical method. Mallards appeared to be resilient to oral single dose MC-LR exposure, and demonstrated clinical and hematological recovery within two weeks. However, lower body conditions for all treated birds, and findings from histopathology were suggestive of longer-term sublethal health impacts. This study is the first to examine the effects of MC-LR exposure in mallard ducks in a laboratory setting. Wild waterfowl are likely to be chronically exposed to MC at low concentrations, and for a prolonged duration. Our preliminary findings warrant investigation of potential sublethal toxic and reproductive impacts of MC-LR in waterfowl, by employing a low dose, long-term oral exposure pattern.

### DETECTION OF OPHIDIOMYCOSIS IN FREE-RANGING SNAKES IN GEORGIA, 2016-2018

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## Abstract

Ophidiomycosis (snake fungal disease) is an emerging disease caused by the fungus Ophidiomyces ophiodiicola. As part of efforts to track the distribution and prevalence of the disease, in addition to assessing overall snake population health, visual encounter surveys of free-ranging snakes were conducted in 39 counties in Georgia from September 1, 2016 through August 11, 2018. A total of 789 individual snakes were sampled, including visual inspection for skin lesions consistent with ophidiomycosis and skin swabbing to detect O. ophiodiicola DNA using qPCR. Sampled snakes represented 34 species, 19 genera, and 4 families; 36.4 % had skin lesions present, 17.4% were positive for O. ophiodiicola DNA, and 85.4% of the qPCR positive individuals had skin lesions. Twenty-three species were qPCR positive, which is the first report of O. ophiodiicola in 12 species. Based on a series of multivariate logistic regression models, species, year, month, sex, and age were significantly associated with ophidiomycosis status. Plain-bellied watersnakes (Nerodia erythrogaster) and Brown watersnakes (Nerodia taxispilota) were positively associated with Ophidiomyces presence; Eastern Indigo snakes (Drymarchon couperi) were positively associated with apparent ophidiomycosis (lesions and O. ophiodiicola present). The year 2018 and the age classes subadult and adult were positively associated with ophidiomycosis, while the month of July was negatively associated. Phylogenetic analysis of qPCR positive snakes showed clustering among crotalids and Nerodia species, while negative cases were clustered in the genera Liodytes, Haldea, and Storeria, which are in the subfamily natricine. Specific analysis of sampled Eastern Indigo snakes indicated that skin lesions were nearly ubiquitous (83.8% of individuals) and all qPCR positive animals had skin lesions. Overall, these results provide additional information about the epidemiology of ophidiomycosis, which has important implications for snake conservation.

# ESTIMATES OF ROOSEVELT ELK CALF SURVIVAL AND FACTORS INFLUENCING MORTALITY IN NORTHWESTERN CALIFORNIA

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### Abstract

Juvenile survival of ungulates is known to be highly variable, yet it is fundamental to understand the dynamics and trends of wildlife populations. Factors influencing calf survival are poorly understood in northwestern California. The objectives of this study were 1) to estimate summer calf survival, annual calf survival, and determine recruitment rates for Roosevelt elk (Cervus canadensis roosevelti) in the area, 2) to evaluate the effects of sex, study area, estimated birth weight, and birthdate on summer calf survival, and 3) to identify factors influencing elk survival by investigating mortalities across all age classes. During 2017 and 2018, our study captured and marked 58 calves and 29 cow elk in Del Norte and Humboldt counties. Preliminary analysis suggested that summer survival was high, with survival rate varying during the first 14 weeks of life. Annual calf survival also appears to have been high, but the analysis is ongoing. A total of 58 mortalities (tagged and opportunistic) was investigated from December 2016 through February 2019, but this work will continue through May 2019. This work is an ongoing collaboration and this information will be used by the California Department of Fish and Wildlife in the management of this game species.

## DEER ATADENOVIRUS A DETECTED IN PERINATAL SAMPLES FROM APPARENTLY HEALTHY DAMS PRIOR TO AND AFTER PARTURITION

Kayla Kauffman<sup>1</sup>, Todd Cornish<sup>1</sup>, Samantha Dwinnell<sup>2</sup>, Matthew Hayes<sup>2</sup>, Katey Huggler<sup>2</sup>, Tayler LaSharr<sup>2</sup>, Kevin Monteith<sup>2</sup>, Brant Schumaker<sup>1</sup>, Myrna Miller<sup>1</sup>

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## Abstract

Young animals are the most susceptible to Adenovirus Hemorrhagic Disease (AHD) and the impacts of AHD on recruitment is poorly understood due to the elusive nature of neonatal ungulates. Mule deer (Odocoileus hemionus) and elk (Cervus elaphus nelson) typically spend the first few days or weeks of their life hiding, which isolates them from potential conspecific reservoirs besides their dam. Dams that are persistently infected and intermittently shedding Deer atadenovirus A (OdAdV-1) could expose their neonates to the virus during that time. Recruitment studies on two mule deer and one elk herd in Western Wyoming provided us the opportunity to research perinatal transmission of OdAdV-1. Prior to this study, all three herds had experienced AHD related mortalities, and in 2015 20% of neonatal mortalities in one mule deer herd were positive for OdAdV-1. We hypothesized that dams were transmitting the virus to their offspring at or near the time of parturition. We collected samples from dams prior to parturition when vaginal implant transmitters (VIT) were placed (n=137), tested the VIT after it was expelled at parturition (n=118), collected samples from the neonates when they were collared (n=151), and collected samples from any mortalities (n=66). The year of this study had a relatively low number of AHD related mortalities (n=8). We were able to detect OdAdV-1 in one elk at VIT insertion and on 6 of the expelled VITs. OdAdV-1 was not detected in any of the neonates at the time of collaring. This represents the first time the OdAdV-1 was detected in an apparently healthy individual in the Rocky Mountain West and demonstrates that offspring may be exposed to OdAdV-1 from their infected dam.

## LONGITUDINAL ASSESSMENT OF DOMOIC ACID EXPOSURE AND RELATIVE HAZARD OF DEATH DUE TO CARDIOMYOPATHY IN SOUTHERN SEA OTTERS (ENHYDRA LUTRIS NEREIS)

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### Abstract

Southern sea otters (Enhydra lutris nereis) are a federally threatened species that has struggled to recover and expand its range in California. The species faces threats from elevated mortality, land-based pathogen pollution, and resource-limitation. Blooms of the marine diatom Pseudo-nitzschia often produce domoic acid, a neurotoxin that causes mortality among marine mammals along the Pacific Coast of North America. Domoic acid toxicosis and cardiac disease are highly prevalent causes of sea otter mortality, and previous research has linked domoic acid exposure to cardiomyopathy in otters. The main objective of this study was to assess individual life histories, behavioral adaptations, and environmental threats underlying cardiomyopathy. Long-term telemetry-based field studies of free-ranging sea otters (n = 205), and necropsies on many of those same individuals (n = 59), permitted a detailed assessment of demographic, behavioral, and environmental characteristics through direct observation and the use of radio-transmitters. This longitudinal study from 1999 – 2017 included otters that were tagged and monitored over much of their lives and examined after death, providing data on age and sex-specific survival, diet, protozoal serology, reproduction, movement, pathology, and causes of death. Additionally, we incorporated temporally and spatially explicit domoic acid data series from environmental monitoring sources. We used a time-dependent, extended Cox regression model to estimate the survival experience of otters with different prey preferences and exposure to domoic acid and compared their hazards of dying from cardiomyopathy. Time-sensitive variables representing the frequency and duration of domoic acid exposure were essential for unraveling the relationship between this toxin and cardiomyopathy. Incorporating foraging behavior provided insight into individual risk and the timeline of cardiomyopathy development in otters. Harmful algal blooms are predicted to increase with climate change, potentially creating an impediment to population recovery. We intend for this modeling framework to optimize science-driven management actions for species conservation.

## IMPLEMENTING ENVIRONMENTAL SAMPLING FOR AVIAN INFLUENZA SURVEILLANCE: DETERMINING WHICH ECOLOGICAL FACTORS PREDICT MATRIX-GENE POSITIVE SAMPLES FROM WETLANDS IN BRITISH COLUMBIA, CANADA

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### Abstract

Avian influenza (AI) is a viral disease that affects domestic poultry world-wide. The economic impact from these outbreaks can be substantial, for example the 2004 poultry outbreak in British Columbia, Canada resulted in euthanasia of > 17 million birds and economic losses at  $^{156}$  million. Avian influenza virus (AIV) can also cause disease in humans and wildlife. Preventing and mitigating impact from AI outbreaks underscores the need for accurate surveillance of AIV. AI outbreak viruses typically arise from strains that circulate in wild waterfowl, thus making wild waterfowl the target of AIV surveillance. However, traditional AIV surveillance has limited effectiveness as sampling in individual wild birds is often biased and may not contain a representative and/or sufficient sample of animals. Many of these limitations can be overcome by surveillance using environmental samples (e.g. wetland sediment), which may contain viruses excreted from multiple birds and multiple species. However, there are still uncertainties regarding the collection, analysis, and interpretation of environmental samples. Our objective is to identify which ecological factors predict the presence of AIV-positive wetland sediment samples. Samples (N = 2,093) were collected (i) monthly from 3 wetlands or (ii) annual from 18 wetlands from the lower mainland, British Columbia, Canada between Sep 2016 to Aug 2018. Samples were analyzed by RT-qPCR for AIV matrix gene positivity. Ecological characteristics included from consideration were pH, salinity, temperature, sampling depth, presence of waterfowl feces, location, date, time, and land use classification. Predictive glmms controlling for clustering by sampling site and wetland were used to explore the relationship between the ecological characteristics and sediment AIV positivity. The results of this study will be important for developing efficient and effective sampling strategies for future AIV surveillance based on environmental samples.

#### USING MULTIVARIATE ANALYSES TO PREDICT STAGE OF INFECTION

Rachel Ruden, James Adelman Iowa State University

### Abstract

Endemic diseases frequently cause seasonal epizootics, but field surveillance often fails to distinguish between individuals in early versus late stages of infection. Refining such predictions could greatly improve our temporal resolution of epizootics in the wild. Here we set out to test whether subtle differences in single assessments of pathology (lesion scores) could accurately predict the stage of infection. Specifically, we used data collected during captive studies of house finches (Haemorhous mexicanus) experimentally-infected with Mycoplasma gallisepticum (MG) to compile two multivariate data sets. In this system, clinical signs (conjunctivitis) are evaluated on a 4-point scale from 0 (none) to 3 (severe). In 2017, we began further characterizing eye pathology with the following descriptors: blepharospasm, crusting, erythema, eversion, exudate, and swelling. We also photographed eyes prior to scoring and applied thin-plate spline overlays using techniques from geometric morphometrics to assign landmarks and compare pathology using shape analysis. Drawing from these two data sets (descriptors and landmarks), we compiled a subset of samples paired by eye score before and after an individual's peak pathology. Community analysis revealed that exudate was the best marker of individuals in early infection, especially at low eye scores (< 1): whereas nearly 40% of eyes exhibiting scores of 1 showed exudate pre-peak pathology, none of these eyes maintained exudate post-peak pathology. We expect shape analysis to reveal similar nuances, as pre-post comparisons will be conducted across 16 landmarks. Our results suggest that multivariate analyses can predict whether individuals are in early versus late infection. This can have important applications for tracking the precise timing of epizootic events.
# DISEASE SURVEILLANCE OF INVASIVE NUTRIA (MYOCASTOR COYPUS) INHABITING WETLANDS IN CALIFORNIA'S CENTRAL VALLEY

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## Abstract

Nutria (Myocastor coypus) are semi-aquatic rodents native to South America that have been introduced into many countries outside their native range where their prolific breeding and feeding habits have resulted in widespread, adverse impacts on wetland ecosystems. Nutria can also be hosts of some zoonotic pathogens and infectious threats to other species. The re-emergence of nutria in the California Central Valley in 2017, nearly 50 years post eradication, necessitates further investigation into the impacts of this species. In this study, we investigate whether nutria in California serve as hosts for infectious agents with spillover risk to susceptible host populations including native wetland species, domestic animals, and humans. We use histopathology, RT-PCR, ELISA and IFA serology, and morphologic identification of ectoparasites to evaluate the presence of bacterial, viral, and protozoal pathogens in 30 nutria dispatched during the first year of a statewide eradication effort. Common histopathologic findings include lymphocytic and granulocytic interstitial pneumonia and hepatitis. We did not detect pathogenic Leptospira spp., Bartonella spp., or Borrelia burgdorferi sensu lato by RT-PCR or Anaplasma phagocytophilum by RT-PCR and IFA serology. Testing for Toxoplasma gondii, Francisella tularensis, Yersinia pestis, Sin Nombre orthohantavirus, Salmonella spp., Giardia lamblia and Cryptosporidium parvum and morphologic identification of ectoparasites is in progress. We use logistic regression analysis to determine which risk factors such as sex, age class, body condition, or county of origin are associated with particular pathogens. This disease surveillance effort serves to identify novel disease presence and risk to other species while elucidating the role of disease risk in future invasive species management. Continued surveillance of nutria and their associated pathogens will inform disease preventive measures and mitigation of potential nutria impacts on native species.

#### POPULATION GENOMICS OF THE INVASIVE TICK, HAEMAPHYSALIS LONGICORNIS

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### Abstract

The Asian longhorned tick, Haemaphysalis longicornis, is native to eastern Asia, but has invaded Australia, New Zealand, and most recently, the U.S. There are several life history traits of H. longicornis that enhance its ability to invade new environments. First, it has a broad geographic and host range including humans, domesticated animals and a wide variety of wild carnivore, cervid, rodent, and avian species. Furthermore, some populations of H. longicornis are capable of parthenogenesis. Therefore, a single female tick introduced to a new area can start a new population. Population genetics have been extensively used to investigate arthropod vectors through estimating gene-flow, dispersal patterns and their potential to spread pathogens. For H. longicornis specifically, determining the genetic structure of tick populations within the U.S. will potentially elucidate critical questions about this invasive tick such as: i) the number of introductions (or lineages) into the U.S. and, ii) population origin of ticks in the U.S. For this study, we obtained restriction-site associated DNA sequences (RADseq) from H. longicornis collected from several U.S. states (AR, VA, NJ, NY, and WV), Australia, and the Sichuan and Hebei provinces in China. We detected 3883 variable loci with 16,168 SNPs among our samples. Our data indicate that H. longicornis has modest amounts of variation within collection locations, but moderate to large amounts of variation among locations. Little variation was detected between NJ and VA, and loci present within these populations are also observed in samples collected from both Australia and China. Samples from AR, NY, and WV have loci that are distinct from other populations potentially indicating multiple introductions. Thus, our data demonstrate that there is considerable genetic variation and structure among the U.S. populations of H. longicornis and supports the use of this technique to investigate origins for this invasive tick.

# PREVALENCE AND CLINICOPATHOLOGIC FINDINGS OF A NOVEL CHLAMYDIALES ORGANISM IN FREE-RANGING RAPTORS ADMITTED TO A VETERINARY TEACHING HOSPITAL IN NORTHERN CALIFORNIA: 2000-2018

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## Abstract

The Chlamydiales are gram-negative, obligate intracellular bacteria known to cause disease in humans and animals. Reports of chlamydiosis in raptors are scarce; recent studies suggest the strain in red-tailed hawks may be a novel species that causes clinical disease. A 2000-2018 retrospective evaluation of medical records at the Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California, Davis was performed to determine the prevalence and clinicopathological findings of Chlamydia spp in free-ranging birds of prey. Sex, age class, location found and admission dates were recorded. Diagnostic tests performed included mucosal and tissue qPCR, EBA and IFA serology, culture, and full necropsy. Animals were classified as negative, positive, or probable based on current guidelines from the National Association of Public Health Veterinarians for diagnosis of chlamydiosis in birds. Of 3,550 raptors admitted, 225 were tested for Chlamdyiales. Fifty nine raptors were determined to be confirmed or probable cases: 55/59 cases were red-tailed hawks (Buteo jamaicensis); 2/59 were Swainson's hawks (Buteo swainsonii); 1/59 was a rough-legged hawk (Buteo lagopus) and 1/59 was a ferruginous hawk (Buteo regalis). Physical findings were generally non-specific and included emaciation, obtundation, and dehydration. Respiratory signs were rare. The most consistent radiographic finding was splenomegaly. A moderate-to-severe leukocytosis characterized by heterophilia, bands, monocytosis and eosinophilia was the most commonly reported CBC abnormality. Full post-mortem examinations were performed on 43/59 animals. Commonly affected organs included the liver (100%), spleen (83%), airsac (80%), heart (70%), lung (61%), and kidney (58%). Juvenile birds were more commonly presented. Most birds presented between December and February. These findings suggest that infection with Chlamydia spp. is a cause of severe systemic disease in free-ranging red-tailed hawks in northern California. More studies are needed to understand the transmission and possible public health implications of this potentially zoonotic agent in free-ranging Buteo raptors.

# ASSOCIATIONS AMONG CLIMATE, STRESS, AND AVIAN INFLUENZA VIRUS INFECTION IN MIGRATORY WATERFOWL

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#### Abstract

Our world is experiencing rapid large-scale environmental changes associated with a changing climate, altered habitats, intensified agricultural practices, natural resource extraction, and increasing emerging infectious diseases. Multiple stressors operating simultaneously may act synergistically in ways that impact wildlife health. Appropriate responses to stressors, mediated by the hormone corticosterone (CORT) can be beneficial in life threatening situations. However, persistent elevations during chronic stress can be energetically costly and have deleterious effects on metabolism, immune function and disease susceptibility, with subsequent impacts on condition, reproduction, or survival.

Waterfowl of the order Anseriformes are natural reservoirs for low pathogenic avian influenza viruses (LPAIV), and generally show no overt signs of disease. Nonetheless, previous studies have demonstrated associations between infection and body condition, timing of migration, or resigning rates, implying that there may be a cost to infection despite the lack of clinical signs.

Our objective was to examine relationships between climate and stress responses in waterfowl, and how they interact to influence subsequent body condition and probability of infection with LPAIV. Over 5000 blue-winged teal were sampled prior to fall migration in the Canadian prairies from 2007 to 2018. Feather CORT (CORTf), which is an integrated measure of corticosterone physiology during the period of feather growth, was measured using commercial ELISAs. Oral and cloacal swabs were tested for presence of AIV using established RT-PCR methods. Climate data was obtained from weather stations closest to sampling sites. Information theoretic approaches to model selection are currently being used to examine associations among environmental variables, CORTf, body condition, and AIV infection, and results will be presented. Understanding relationships among climate, stress, and AIV infection in waterfowl will be increasingly important, to help understand how a rapidly changing climate may ultimately impact the dynamics of LPAIV in waterfowl populations.

#### DEFINING PATHOGENS AND DISEASE OF MUSKRATS (ONDATRA ZIBETHICUS) IN PENNSYLVANIA

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## Abstract

Declines in muskrat (Ondatra zibethicus) harvest have been observed throughout their North American range. The exact cause of the declines is unknown, but potential theories include habitat loss or degradation, predation, and disease. Minimal recent data exists on muskrat diseases. To address this knowledge gap, we employed a multifaceted approach to define diseases and pathogens of muskrats, including: 1) retrospective study of published data on muskrat diseases in North America; 2) review of diagnostic data on muskrats in the Eastern United States; 3) active surveys of diseases and pathogens of trapper-killed muskrats throughout Pennsylvania, and 4) a telemetry study to characterize survival of muskrats in Pennsylvania. The latter two components are currently in progress. Google Scholar and Web of Science <sup>™</sup> were searched to find existing scientific literature related to muskrat exposure to diseases, parasites, or contaminants. All muskrat diagnostic records (n=27) from the Southeastern Cooperative Wildlife Disease Study were reviewed for cause of disease. Over 900 trapper-harvested muskrat carcasses were collected during 2019 from throughout Pennsylvania and examined for lesions or parasites and were tested for specific diseases of historic significance (e.g. Tyzzer's disease and Tularemia). A wide-diversity of diseases, parasites and contaminants were identified through the retrospective analyses, with the most common infectious diseases included Tularemia, Tyzzer's disease, Echinostomiasis, and Cysticercosis. Similarly, the most common diagnoses in muskrats from the Eastern US were Tyzzer's disease (15%) and Cysticercosis (11%). These data provide valuable insights into diseases and pathogens of muskrats in North America and are be used to guide targeted surveys on trapper-harvested muskrats and future research efforts.

#### THE NASAL MICROBIOME OF FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN FLORIDA

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## Abstract

The farmed white-tailed deer (Odocoileus virginianus) industry is a growing agricultural system of economic importance across North America and in the state of Florida, with over 300 licensed facilities present across the state. Farmed White-tailed deer often suffer from respiratory infections, making the identification and characterization of pathogenic bacteria extremely important for a correct treatment. Although such an extensive industry, limited studies have been conducted to characterize the normal microbiota of healthy farmed deer compared to other ruminant livestock species, which have benefitted in management and herd health. Nasal swabs collected in RNALater media, along with other diagnostic samples, were collected from the 2017 fawn crop of a private farm on three separate occasions; day of birth, time of weaning (3 months), and nearing adulthood at 9 months. Overall, 8 animals survived the year and were healthy at the time of each sampling event providing 24 total samples. DNA was extracted and sequenced using the Illumina MiSeq platform. Sequence data was processed using Mothur software in order to determine relative abundance and temporal differences of the nasal microbiota to the genus level. The number of sequences per sample ranged from 98,181 to 155,978 with an average of 124,987 sequences per sample. A total of 658 OTU's were reported across all 24 samples. Samples were found to be consistent with the dominant phyla in other ruminant animals, such as feedlot cattle and horses. However, data obtained from this study will be among the first for farmed White-tailed deer. These findings and future research will provide an accurate baseline of healthy animals, as well as comparative data when coupled with microbiome characterization of animals suffering from viral or bacterial infections. This information will provide farmers and veterinarians with a better understanding of how to properly manage and treat future respiratory illness.

# HIGH VECTOR-BORNE HAEMOSPORIDIA PREVALENCE IN EASTERN SANDHILL CRANES (ANTIGONE CANADENSIS) OVER TWO DECADES

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## Abstract

Haemosporidians are a diverse group of vector-transmitted blood parasites that include the agents of human and avian malaria. In birds, the impacts of haemosporidian infection can range from asymptomatic to acute mortality or population-level impacts on fecundity and survival. These parasites have been found in Sandhill Cranes (Antigone canadensis), a migratory species that may serve as a sentinel for understanding health threats to the endangered Whooping Crane (Grus americana). We used molecular techniques to compare prevalence and diversity of Haemosporidia in wild Sandhill Cranes from the eastern flyway population that migrates annually between Wisconsin and the southeastern US. Using archived blood samples collected over a twenty-year period (1997-2017), we amplified the mitochondrial cytB gene of *Plasmodium* and *Haemoproteus*. Phylogenetic analysis is being conducted to identify parasite lineages. Overall the infection prevalence in birds (n=175) was 71.4%, with no difference across early versus late time period sampled (P=0.43) or between sexes (P=0.53). Infection prevalence in young (hatch year) birds (80.7%; n=109) was significantly greater than that of older birds (66%; n=66; P=0.0009); this may be related to the immune status or relative lack of anti-vector defense of immature individuals. In at least one recaptured individual, PCR and sequencing revealed evidence of co-infection with both a *Plasmodium* sp. and a *Haemoproteus* sp. The baseline data gathered from this study will contribute to our understanding of the factors that influence Haemosporidian prevalence in Sandhill Cranes over time and inform directions for future epidemiologic studies and conservation efforts.

# CAUSE OF DEATH IN FLORIDA FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) DURING 2017 - 2018

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#### Abstract

White-tailed deer (Odocoileus virginianus) farming is an emerging agricultural industry in Florida. Bacterial infections and viral hemorrhagic diseases cause high mortality in fawn and yearling deer, and are a source of significant production loss among Florida deer farmers. Before management can be improved and properly implemented, the causality of death in the farmed herds must be determined. The University of Florida Cervidae Health Research Initiative (CHeRI) provides a diagnostic service to Florida deer farmers to determine and monitor the proportion of farmed white-tailed deer that have died from bacterial infections, hemorrhagic disease-causing viruses, or other causes of death. From 2017 to 2018, participating Florida ranches provided recently deceased farmed white-tailed deer for necropsy or shipped tissues for analysis by the CHeRI diagnostic program. Both necropsy and owner-sampled tissues were tested for hemorrhagic disease using qPCR, and were subjected to additional microbial culture, histopathology analysis, and parasite identification as necessary to determine probable cause of death. Of the 128 deceased farmed white-tailed deer sampled in 2017, 39% of deaths were associated with bacterial infection, 44% were attributed to viral hemorrhagic disease, and 17% of deaths were due to other or undetermined causes. Of the 152 animals sampled in 2018, 40% of deaths were attributed bacterial infection, 49% were associated with hemorrhagic disease virus, and the remaining 11% of animals sampled died of other or unknown causes. Viral hemorrhagic diseases and bacterial infections are major sources of mortality in farmed white-tailed deer. These data provide white-tailed deer farmers with insight on how to improve management practices, thereby improving herd health and reducing mortalities.

#### A SCOPING REVIEW OF WILDLIFE HEALTH AND DISEASE SURVEILLANCE LITERATURE IN CANADA

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## Abstract

Wildlife surveillance is necessary for early detection of emerging health threats and appropriate response. Because of growing recognition of the value of surveillance, we set out to examine the extent and range of published Canadian wildlife surveillance data. We conducted a scoping review, using the Arksey and O'Malley framework, to identify and summarize the Canadian wildlife health and disease surveillance literature including the identification of commonly described limitations. A search was conducted in three electronic databases to identify Canadian wildlife surveillance data published between 2007 and 2017. The search identified 508 research papers. All studies were independently screened and characterized by two reviewers. The reviewers met to ensure consistency and resolve any discrepancies. Preliminary results suggest that there is a clear trend in the published surveillance literature towards active surveillance of avian species carried out by academic institutions for the purpose of detecting or quantifying a particular pathogen or disease. Whether this trend is representative of the nature and scope of existing wildlife surveillance programs in Canada, a function of differing capacity and motivation for publication between surveillance programs, or some combination of the two, it highlights that marked knowledge gaps exist within the published literature. Additionally, important questions are established about how wildlife surveillance data is communicated, whether surveillance objectives impact what data is published, and whether publication influences who accesses the data and how it is used.

# LEPTOSPIROSIS IN THE CHANNEL ISLAND FOX: A LONG-TERM STUDY TO ASSESS THE ORIGIN, SPREAD AND IMPACT OF LEPTOSPIROSIS IN THE CHANNEL ISLAND FOX

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#### Abstract

In 2010 an outbreak of Leptospirosis caused by Leptospira interrogans serovar Pomona was detected in the reintroduced population of Channel Island fox on Santa Rosa Island (Urocyon littoralis Santarosae). Here we combine 15 years of sample collection, mark-recapture and telemetry data along with archived pre-decline samples and samples from other wildlife species to assess the origin, spread and impact of Leptospira in the Channel Island fox on Santa Rosa Island. We have discovered that Leptospira was present on Santa Rosa Island in, the island foxes prior to their population crash in the 1990's, though did not persist in the captive foxes in the on-island captive breeding facility that all remaining 15 foxes were placed in. However, during the reintroduction of island foxes back into the wild on Santa Rosa Island naïve captive born foxes became infected immediately upon release, with evidence showing that the bacteria was likely harbored in the island spotted skunk (Spilogale gracilis) while the foxes were in captivity. Using the thousands of blood and urine samples collected during the 15 years of trapping post reintroduction we have been able to track the prevalence of the bacteria in the foxes over time and learn about the transmission dynamics including force of infection, duration of-shedding, titer dynamics, variation in the presence of maternal antibodies and cross-reactivity. Using the data from the long-term telemetry and mark-recapture data we are learning about the impact of Leptospira on the survival and reproduction in the Channel Island fox.

# CHARACTERIZATION OF A NOVEL ORTHOREOVIRUS SOLATED FROM DEAD STRANDED HARBOR SEALS IN WASHINGTON STATE, USA

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## Abstract

From October 2007 to July 2008, 24 harbor seals (Phoca vitulina) stranded dead along the shores of Puget Sound, Washington State, USA. The carcasses were necropsied and selected samples were processed for histopathology and diagnostic testing including virus isolation. Isolates displaying identical cytopathic effects (CPE) in Vero.DogSLAMtag cells were recovered from brain, lymph node, lung, spleen, heart, skin, kidney, and abdominal cavity fluid of four male weaned pups and an adult male seal (5/24). Infected Vero.DogSLAMtag cells were processed for transmission electron microscopy revealing arrays of non-enveloped, spherical virus particles (~70 nm in diameter) within the cytoplasm, consistent with members of the family Reoviridae. RNA was extracted from the same isolate using a QIAamp Viral RNA Mini Kit and a cDNA library was generated for sequencing on an Illumina MiSeq sequencer. The complete coding sequences of the segmented genome of a novel reovirus was determined. Genetic and phylogenetic analyses revealed the isolate represents a new strain of the species Mammalian orthoreovirus within the genus Orthoreovirus (hereafter referred to mammalian orthreovirus 5; MRV-5). MVR-5 represents the first mammalian orthoreovirus characterized from dead stranded harbor seals. A study conducted in 1989 reported the presence of reovirus-like virus particles in a single sample from an emaciated harbor seal pup in Washington State, USA; however, the virus was not isolated. In 2005, a novel Avian orthoreovirus was isolated from tissues of an aborted Steller sea lion (Eumetopias jubatus) fetus in Canada. In the present study, gross and microscopic lesions observed in harbor seals from which MRV-5 was isolated were inconsistent, and thus, the role MRV-5 in disease (if any) could not be determined. Further research is needed to determine the prevalence, pathogenicity, and host range of orthoreoviruses in pinnipeds.

## DO FREE RANGE CHICKENS EQUAL FREE-RANGE PATHOGENS?

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## Abstract

Backyard chickens continue to gain in popularity throughout the U.S, and so does the incidence of poultry-associated pathogens. The current outbreak of virulent Newcastle disease virus in California is the third vNDV epidemic since 1971; however, risk management continues to neglect the ecology of NDV. We performed two field experiments to determine how backyard chicken husbandry and feeding influence the transmission of NDV to periurban songbirds. Using a live NDV vaccine, the LaSota vaccine virus, we inoculated chickens living under two conditions at two farms in Athens, Georgia. Flock S was maintained in a coop both day and night, whereas Flock L was maintained as a flock of free-ranging chickens during the day. Prior to inoculating chickens, wild birds were mist-netted, banded, blood sampled, swabbed, and tested for the presence of wild-type Newcastle disease virus. Wild bird capture continued for 21 days post-inoculation to determine which species were positive for LaSota. We also performed a supplemental feeding trial at the same sites to identify which wild bird species were most likely to enter coops to feed opportunistically and become infected by the LaSota vaccine. Our preliminary results suggest that cooped chickens attract more songbirds in higher densities and are more likely to infect a greater number of wild birds due to a concentrated viral load. Serology of our experimental chickens indicate a strong, immunogenic response in to the vaccine between days 4 and 11-post inoculation. Observational data suggests that small common bird feeder birds such as Northern Cardinals (Cardinalis cardinalis), Tufted Titmice (Baeolophus bicolor), and Carolina Chickadees (Poecile carolinensis) followed by ground foraging species, such as Chipping Sparrows (Spizella passerine), demonstrated behaviors such as visitation frequency, consuming chicken food, and time spent in the coop that puts them at the greatest risk of being infected by a chicken pathogen.

#### NOTOEDRIC MANGE IN SMALL INDIAN MONGOOSE (URVA AUROPUNCTATA) FROM HAWAII

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## Abstract

Mongooses (Urva auropunctata) were introduced to Hawaii in an effort to combat rodent infestation of cane sugar plantations, and they have subsequently become an invasive species. As local mongoose numbers and range increase, their ecological effects as predators or disease carriers may become more important. We captured individual mongooses that showed signs of mange, and we attempted to identify the mites present in skin samples from them. Mites were separated from a mongoose sample under a dissecting microscope. DNA extracted from 8 mite specimens was subjected to PCR reactions specific for the suspected mange agent, Notoedres cati. Agarose gel electrophoresis was performed on the post PCR products and each sample was PCR positive. Subsequent DNA sequences from these samples were matched to known sequences in the NCBI database. The sequences of the mongoose mite samples were highly similar to those of Notoedres cati ribosomal RNA, with homologies ranging from 86%-96%. This suggests that the mites infesting Hawaiian mongooses are Notoedres cati or a highly similar mite species. This finding is notable because mongoose populations living within urban areas could be potential sylvatic reservoirs for notoedric mange in feral and domestic cats.

# EVALUATING THE USE OF GUIDE AND OUTFITTER KNOWLEDGE AND SAMPLING TO INFORM WOODLAND CARIBOU (RANGIFER TARANDUS CARIBOU) CONSERVATION

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#### Abstract

The Northern Mountain Population of woodland caribou (NMPC; Rangifer tarandus caribou) in northwestern British Columbia is of great cultural, economic, and ecological significance to the local First Nations, resident and guided hunting industry, and Canadians in general. This population faces the increasing threats of a changing landscape and climate, and considerable concerns have been raised by local land-users regarding declining numbers and erratic herd behaviours. Despite this, there is insufficient information on the NMPC to understand current health status, predict future population trends, and ultimately support informed and adaptive management.

The goal of this study is to explore novel ways to assess and monitor remote wildlife populations in partnership with outfitters and guides. We use a multi-disciplinary approach, bridging harvest-based wildlife sampling and traditional scientific methods with local knowledge from guides and outfitters to arrive at a locally-based caribou health surveillance framework. We collected local knowledge from 16 guides and outfitters identified as 'caribou experts' using systematic in-depth individual interviews while also incorporating semi-quantitative participatory activities such as mapping, timelines, and proportional piling. Group sessions reflecting the 7 study herds with enhanced participant pools will allow for participant validation and data triangulation. Using thematic analysis, qualitative data will be summarized and presented back to stakeholders. We analyzed biological samples from 63 harvested caribou over the 2016 to 2018 hunting seasons for various markers of individual and population health, including body condition, bacterial, viral, and parasitic pathogen-exposure, contaminant and trace mineral levels, demographics, and stress.

Using a concurrent-triangulation mixed methods approach, both guide-outfitter local knowledge and scientific data from harvest-based sampling will cooperatively inform on the health, status, and trends of the NMPC. This research shows significant promise of establishing a comprehensive, locally-based, caribou health surveillance framework that can successfully be applied to other remote species, populations, and systems.

# CHARACTERIZATION OF PSEUDOGYMNOASCUS DESTRUCTANS CONIDIAL ADHERENCE TO EXTRACELLULAR MATRIX AND ITS ASSOCIATION WITH FUNGAL SECRETED PROTEASES IN WHITE-NOSE SYNDROME OF BATS

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## Abstract

Pseudogymnoascus destructans (Pd) is the fungal pathogen of white-nose syndrome (WNS), which has caused dramatic mortality of hibernating bats. The fungus causes erosion and ulceration of the epidermis, especially on the patagium, muzzle and ears. Typically, fungal adherence to host cells and extracellular matrix (ECM) provides the critical step to initiate the infection. Previous studies have proposed that Pd serine proteases (PdSPs) and a Pd metalloprotease (Pd Asp f 2) could contribute to Pd virulence. Therefore, the main goal of this study was to characterize the process of fungal adherence to ECM and investigate the roles of PdSPs and Pd Asp f 2 in association with this initial contact between fungal conidia and ECM. In vitro adherence assays to ECM were performed on laminin or fibronectin-coated 96-well plates at 12°C. Fungal conidia were added to each well coated with different concentrations of ECM to determine the appropriate ECM concentration. Separately, we also performed the adherence assay to ECM and varied the incubation time to determine the maximal binding time point. The number of conidia remaining adherent after washing were observed by a phase-contrast microscopy. The association of secreted proteases was evaluated by differential gene expression analysis from qRT-PCR. The results showed that Pd conidia binded to laminin and fibronectin in the dose and time-dependent manner. During initial contact with ECM, we observed changes in secreted protease genes expressions, indicating that Pd secreted proteases may be differently regulated during the adherence process. Our adherence assay to ECM demonstrates that Pd conidia can bind to ECM in vitro. This study has provided us an in vitro model for studying the initial interaction between host tissues and fungal conidia in bat WNS, which can be used to investigate underlying molecular mechanisms in future studies.

# CLIMATE CHANGE AND INTENSIFYING WILDFIRES: EXPLORING INTEGRATIVE APPROACHES TO TREATING BURNED WILDLIFE IN CALIFORNIA

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## Abstract

Three black bears (Ursus americanus) were rescued by the California Department of Fish and Wildlife (CDFW) during the Thomas Fire in December 2017 and the Carr Fire in August 2018. All bears had severe full-thickness burns to their paws and were significantly incapacitated; following rescue, they were treated at the CDFW Wildlife Investigations Laboratory (WIL). CDFW WIL and the Integrative Medicine Service at the University of California, Davis Veterinary Medical Teaching Hospital employed novel therapies to manage pain and accelerate healing, including specially formulated topical medications, cold laser therapy, pulsed electromagnetic field therapy, and acupuncture. Additionally, sterilized tilapia skin was applied directly to the burns to establish a "biologic bandage" on each paw. Tilapia skin bandages have previously been used to successfully treat human burn patients in Brazil, but to the authors' knowledge, this is the first time its use on animals has been reported. In addition to providing protection against mechanical and infectious agents, as well as decreasing pain by covering exposed nerves, collagen and marine biopeptides in the tilapia skin promoted wound healing and tissue regeneration. All bears were successfully treated and returned to the wild within 4-6 weeks of their rescue. Two of the three bears survived for at least 7-10 months since their release, while the survival status of the third bear is unknown due to tracking device failure. By employing novel therapies to accelerate healing, the time injured wildlife spends in captivity is decreased, thereby minimizing habituation and maximizing an animal's chance of returning to the wild. With worsening climate change and the ever increasing threat of wildfires, treatment of burned wildlife will become even more of a pressing issue in the future.

# SARCOPTIC MANGE IN CHILEAN MAMMALS. THE IMPORTANCE OF ASSESSING EMERGING THREATS IN WILDLIFE AT ADEQUATE TEMPORAL AND SPATIAL SCALES TO PROMOTE ACTION

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## Abstract

Cases of sarcoptic mange have been anecdotally reported during the last decade in Chilean wild mammals. This disease can have devastating effects on biodiversity, including the extinction of species, and there is a concern that it may be spreading in this country. However, Chile does not have a wildlife disease surveillance program to undertake assessments of diseases at relevant scales to investigate this hypothesis. To fill this gap, we collected information on the observation of wild mammals with alopecia during the last 15 years in Chile using three methods: park rangers, citizen reports, and print media. We used hair loss as a proxy for sarcoptic mange as it is a typical sign of this disease, is easy to observe, and can be documented photographically. Further, we assessed the relationship between this sign and sarcoptic mange by diagnosing *Sarcoptes scabiei* in foxes with alopecia. Our results indicate that alopecic mammals are being observed more frequently, involving a larger number of species and that their presence has expanded during the study period. The number of protected areas with sightings increased from 5 to 13 in the last 15 years and now cover the entire country. In addition, citizen reports confirm the presence of affected animals outside protected areas. While cases were reported in nine wild and two domestic species, most of the sick animals are foxes (Lycalopex sp.) endemic to the neotropics. Diagnostic confirmation of S. scabiei showed that alopecic mammals present a very high probability of being infested with this mite. Our results encompassing more than 10 years and at the country level, strongly suggest the spread of sarcoptic mange in wild mammals and warrant the urgent implementation of a national wildlife health surveillance program and the convening of an inter-institutional working group to address this emerging and potentially catastrophic disease.

# DEVELOPMENT AND VALIDATION OF QUANTITATIVE PCR ASSAYS TO MEASURE CYTOKINE TRANSCRIPT LEVELS IN THE FLORIDA MANATEE (TRICHECHUS MANATUS LATIROSTRIS)

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#### Abstract

Cytokines have important roles in the mammalian response to viral and bacterial infections, trauma, and wound healing. Because of early cytokine production after physiologic stresses, the regulation of messenger RNA (mRNA) transcripts can be used to assess immunologic responses before changes in protein production. To detect and assess early immune changes in endangered Florida manatees (Trichechus manatus latirostris), we developed and validated a panel of quantitative PCR assays to measure mRNA transcription levels for the cytokines interferon (IFN)- $\gamma$ ; interleukin (IL)-2,-6, and-10; tumor necrosis factor- $\alpha$ ; and the housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and  $\beta$ -actin (reference genes). Assays were successfully validated using blood samples from free-ranging, apparently healthy manatees from the east and west coasts of central Florida, US. No cytokine or housekeeping gene transcription levels were significantly different among age classes or sexes. However, the transcription levels for GAPDH, IL-2, IL-6, and IFN- $\gamma$  were significantly higher (P<0.05) in manatees from the east coast of Florida than they were from those from the west coast. We found IL-10 and  $\beta$ -actin to be consistent between sites and identified  $\beta$ -actin as a good candidate for use as a reference gene in future studies. Our assays can aid in the investigation of manatee immune response to physical trauma and novel or ongoing environmental stressors.

# EFFECTS OF INCUBATION TEMPERATURE ON INTESTINAL LENGTH IN LOGGERHEAD SEA TURTLE (CARETTA CARETTA) HATCHLINGS FROM BOCA RATON, FLORIDA

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## Abstract

Incubation temperatures affect sex, size, and survivability of hatchling marine turtles. After hatching, contents of the yolk sac are slowly absorbed into the intestine providing nutrition for several days while the hatchling digs out of its underground nest, locates the ocean and swims to distant nursery areas. Environmental temperatures are rising at sea turtle hatching sites worldwide. Incubation temperatures during the middle third of incubation are key as that time is important for intestinal growth and development. Anecdotal observations of dead-in-nest turtles and turtles that died shortly after hatching from hot nests, suggested that intestinal tracts were shorter than normal. Therefore, we hypothesized that incubation temperatures had a direct effect on the percentage or "length fraction" of gastrointestinal tract composed of intestine. We also hypothesized that the length fraction decreases as incubation temperatures increase. To test these hypotheses, we evaluated the effect that incubation temperatures have on the intestinal length and length fraction available to absorb yolk sac nutrition in the immediate post-hatch period. We necropsied 41 loggerhead sea turtle (*Caretta caretta*) hatchlings from Boca Raton, Florida that incubated in situ, in relocated nests in the beach, or in nests moved to lab incubators. The carapace length, total alimentary length, and intestine length were measured, and intestine length fraction was calculated. The mean temperature for the middle third of incubation duration was negatively correlated with intestine length. The mean temperature for the middle third of incubation and the maximum temperature experienced in the initial third of incubation were negatively correlated with intestine length fraction. Shorter intestinal length fractions at higher incubation temperatures could mean less intestinal surface area and less enteral absorption of the yolk sac contents, leading to negative energy balance and subsequent death. These data provide a possible explanation for decreased survival in hatchlings incubated at higher temperatures.

# NORTH VS. SOUTH: DEPLETED AIRWAY MICROBIOTA OF SOUTHERN HUMPBACK WHALES AT THE END OF MIGRATION MAY SUGGEST COMPROMISED HEALTH

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### Abstract

**Background**: With a journey that can exceed 5,000 miles (8,000 km), humpback whales (*Megaptera novaean-gliae*) undertake the longest seasonal migration of any mammal. On their journey they spend several months fasting. Prolonged fasting, coupled with high-energy behaviours associated with breeding and progressively depleting energy stores, are likely to impact the whales' health.

**Objectives**: The composition of airway microbiota is linked to overall health in humans and horses. Airway microbiota may provide a window into the health status of free-ranging whales. To determine if there were changes in the microbial communities, as a response to the physiological challenges over migration, we compared the microbiota of exhaled-breath-condensate ('blow') of east Australian humpback whales (HW) on their northern versus southern migrations.

**Methods**: We compared the blow microbiota composition of 20 whales on their northern migration (HW-NM) with 20 whales, of the same population, on their southern migration (HW-SM). We analysed the bacterial DNA in the blow by barcode tag sequencing using bacterial 16S rRNA gene.

Results: The blow microbiota of HW-NM and HW-SM were significantly different. The HW-NM had a richer and more diverse microbiota with a much larger number of core taxa (defined as being present in 60, 80 or 100 per cent of whales of one group).

**Conclusions**: We hypothesize that the relatively rich and diverse blow microbiota of whales on their northward migration reflects a state of 'healthy' physiology. At the time of sample collection, the whales had not commenced their fast, so presumably were in 'nutritional prime'. Whereas, the comparably low diversity and number of core taxa in the blow of the

whales returning to their feeding grounds might reflect compromised health due to the depletion of energy stores. This is the first evidence that the blow microbiota of whales might be directly correlated to overall health.

# ANTIBIOTIC RESISTANCE GENES AS INDICATORS OF ANTHROPOGENIC IMPACT IN WILD GUIGNA (LEOPARDUS GUIGNA) HABITAT, CHILE: THE FIRST DETECTION OF MECA IN SOUTH AMERICAN WILD FELIDS

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## Abstract

Antimicrobial resistance is a global emerging Public Health issue whose presence and impact in wildlife is widely unknown. Antimicrobial resistance genes (ARGs) are considered environmental contaminants, suitable to evaluate the degree of anthropic impact on wildlife and the environment. The guigna (Leopardus guigna) is the smallest wild felid in the Americas, endemic to central-southern Chile shrublands and forests, listed as vulnerable by the IUCN Red List (2015). We used wild guignas as sentinels for the presence of ARGs in anthropized and pristine areas across their entire distribution range in Chile. We evaluated fecal samples from 51 wild guignas, collected between 2009-2018. Real-time PCR essays were employed to detect and quantify 22 selected ARGs in their fecal microbiome: tet(A), tet(B), tet(Y), tet(K), tet(M), tet(Q), tet(S), tet(W), sull, sull, str, aadA, catl, catll, erm(B), erm(F), qnrB, qnrS, bla<sub>TEM</sub> bla<sub>CTX-M</sub>, mcr-1 and mecA. All animals (100%) were positive to at least one ARG. Tetracyclines (88.2%) and beta-lactams (68.9%) were the most prevalent ARG families, with tet(Q) (60.8%) and tet(W) (60.8%), and blaTEM (66.7%) as the most prevalent ARGs. Multi-resistant profiles (positive to more than two different ARG families) were observed in 43% of the guignas. Statistically significant differences were found between anthropized and pristine areas regarding to tet(Q) prevalence (p=0.014), tet(W) prevalence (p=0.0037) and quantification (p=0.004), tetracycline-family prevalence (p=0.027), and multi-resistant profiles prevalence (p=0.043). Two animals from anthropized landscapes were positive to mecA. a gene associated with Methicillin-resistant *Staphylococcus aureus*, a relevant cause of nosocomial infections worldwide. This is the first study on ARGs in wild felids from Chile and the first detection of mecA in South American wild felids. We observed an association between the landscape anthropization degree and ARGs prevalence, confirming ARGs are important indicators of wildlife exposure to human activities/presence in Chile, with a widespread distribution, even in pristine ecosystems.

#### ESTABLISHING TISSUE CULTURE CELL LINES FROM REPTILES AND AMPHIBIANS

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#### Abstract

Tissue culture cell lines are a critical reagent for the isolation and characterization of obligate intracellular pathogens. While a wide variety of cell lines are available from humans and domesticated mammals, cell lines from wildlife species, particularly reptiles and amphibians, are extremely limited. As part of an ongoing project to increase the availability and access to reagents for wildlife disease research, we are establishing continuously dividing cell lines from species of herpetofauna. Cell lines are derived from primary tissues collected at the time of necropsy, portions of surgical biopsies, or from embryonic tissues. Primary cell growth was routinely observed from a variety of tissues, including heart, spleen, kidney, skeletal muscle, liver, and gonad. Continued selection for stable, dividing cells of homogenous morphology was achieved through passage in growth conditions determined optimal for the Class of the origin host. Aliquots of cell lines were routinely archived in liquid nitrogen to create low passage stocks. After 10-12 serial passages characterized by stable, predictable growth of cells representing a single cellular morphology, the cell line was considered established and aliquots of working stock cells were archived in liquid nitrogen. For lines characterized by mixed morphology cells, continued passage for selection of single morphology populations was continued. Final cell line characterization includes confirmation of host origin by sequencing of the cytochrome oxidase 1 gene, and immunohistochemical characterization when possible. At the time of submission, cell lines have been successfully established of various tissues from a variety of reptile species, including snakes (n=6), turtles/tortoises (n=4), crocodilians (n=3), and lizards (n=2). Additional cell lines from other species are currently in development. Cell lines will be cataloged in a publicly accessible virtual database to permit and promote the dissemination of these reagents to improve disease diagnostics and characterization of captive and free-ranging wildlife species.

# GENERATION OF ANTIBODY RESPONSE FOLLOWING ORAL VACCINATION WITH MICROENCAPSULATED BACILLUS ANTHRACIS STERNE STRAIN 34F2

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#### Abstract

Anthrax (Bacillus anthracis) is a zoonotic disease endemic to environments worldwide. Spores, the dormant form of the bacteria, can survive for decades in nature's harshest environments. Outbreaks are common in free-ranging livestock and wildlife, thus making anthrax an economically and ecologically important disease. The current vaccine for livestock and wildlife is a suspension of Bacillus anthracis Sterne Strain 34F2 spores in saponin, however it is only available as a subcutaneous injection which is an impractical method of prevention for wildlife. The goal of this study is to develop an oral vaccine that will generate a protective immune response. Prior research has shown that a microencapsulated vaccine against *Brucella abortus* resulted in a higher antibody titers than an un-encapsulated counterpart suggesting that a controlled release vehicle may have similar benefits for an anthrax vaccine. Sterne strain 34F2 spores were encapsulated in alginate and coated with a proteolysis resistant protein. Another vaccine formulation included the same protein within the capsule core as well as on the external shell. Capsule and spore stability at varying pH's were assessed in vitro. Capsule exposure to pH 2 results in premature release of spores from the capsules while spore exposure to pH 2 results in spore death, confirming that protection from the gastric environment is of main concern when producing an oral vaccine. Post vaccination IgG and IgA titers in serum and fecal samples indicated that both types of capsules induced anti-anthrax specific responses following oral and subcutaneous vaccination, suggesting that further optimization of this novel vaccine formulation may result in a reliable oral vaccine that will conveniently and effectively prevent anthrax in wildlife populations.

# PREVALENCE OF MULTI-DRUG RESISTANT ESCHERICHIA COLI IN FREE-RANGING CALIFORNIA CONDORS (GYMNOGYPS CALIFORNIANUS)

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## Abstract

Despite the success of California condor (Gymnogyps californianus) reintroductions, health threats to free-ranging condors persist, especially exposure to high levels of lead from scavenging carcasses. As obligate scavengers, California condors are also likely exposed to antimicrobial resistant bacteria in scavenged carcasses; however, little is known of how differences in foraging patterns across the condor's current range alter their exposure to, and potential spreading of, antimicrobial resistance. Our objectives were to determine the prevalence of multidrug resistant Escherichia coli in free-flying California condors in Southern California, Big Sur, and Pinnacles National Park to examine factors potentially associated with multi-drug resistance, including age, sex, prior treatment for lead poisoning, and dependence on proffered feeding. From July to November 2010, 51 free-ranging California condors were swabbed (cloacal) during routine capture for management. Antimicrobial susceptibility testing using broth microdilution for resistance to 15 antibiotics in 6 drug classes (aminoglycosides, beta-lactamases, chloramphenicol, tetracyclines, fluoroquinolones, and potentiated sulfonamides) was performed on one to three E. coli isolates per sample. Condors were considered positive for multi-drug resistant E. coli if at least one isolate was resistant to one or more antibiotics from three or more drug classes. We detected multidrug resistant *E. coli* in 33% (17/51) of condors sampled. Results of multivariable regression modelling suggest that birds with greater dependence on proffered food within 30 days prior to sampling had lower odds of multidrug resistance. Individual variation in drug resistance likely reflect the range of food sources available in the California condor range. Further examination of patterns in antimicrobial resistance in condors will improve our understanding of the potential for obligate scavengers to serve as an indicator for antimicrobial resistance in the environment and will help to optimize surveillance and antimicrobial therapy in this endangered species.

# THE EFFECT OF DEXAMETHASONE ON HEMATOLOGICAL PROFILES AND HISTOLOGICAL APPEARANCE OF IMMUNE SYSTEM ORGANS IN HOUSE FINCHES (HAEMORHOUS MEXICANUS) INFECTED WITH MYCOPLASMA GALLISEPTICUM

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## Abstract

The emergence and rapid spread of *Mycoplasma gallisepticum* (MG) in house finches (*Haemorhous mexicanus*) across eastern North America since 1994 resulted in one of the most closely monitored epornithic events to date. This may be attributed to the striking conjunctival lesions that allowed for data collection through a citizen science program, conducted by the Cornell Laboratory of Ornithology. Experimental studies have reproduced disease and field studies have demonstrated the effects of mycoplasmosis on house finch survival. Less explored is the role of stress in the development of mycoplasmosis. In other bird species, the administration of dexamethasone, a synthetic, long-acting glucocorticoid, has induced immunosuppression with increased susceptibility to infectious agents and involution of immune organs. Hematologic alterations of lymphopenia and heterophilia have also been demonstrated. Dexamethasone was used here to induce stress-like immunosuppression in house finches following recovery of an experimental MG infection. Eighteen birds were injected subcutaneously with either dexamethasone (experimental group, n=7) or saline (control group, n=8) daily for 8 days. Blood smears were prepared at day 0, 4, 8 and 9 of treatment, and birds were then euthanized. Necropsies and histopathologic examination were performed. Sections of spleen were graded on the histologic presence of follicles and CD3 immunoreactivity. White blood cell differential counts were performed on all blood smears. Results demonstrated a difference in splenic lymphoid tissue, with a decreased number of lymphoid follicles in dexamethasone-treated birds. Dexamethasone induced a relative lymphopenia and heterophilia, severe on day 4 and almost absent by day 9. An increase in intestinal coccidiosis in the dexamethasone-treated birds was also noted. Preliminary results suggest that dexamethasone is capable of inducing immunosuppression in house finches, likely comparable to that of stress-induced immunosuppression, and this may result in recrudescence of MG infection, as well as comorbidities such as intestinal coccidiosis.

#### CHLAMYDIA IN AUSTRALIAN PARROTS AND FREE-RANGE POULTRY

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## Abstract

Chlamydia is a diverse genus of bacteria affecting both animals and humans. Chlamydia psittaci commonly infects parrots but can also cause disease in other bird species, and in humans, where it can cause severe respiratory symptoms and even death. Chlamydia gallinacea is a more recently characterised species, identified in poultry. The effects of C. gallinacea on hosts are unclear although it may similarly present a wildlife and zoonotic health risk. We investigated: [1] the prevalence of *Chlamydia* infection in 5 wild Australian parrot species, and [2] how prevalence compares between wild parrots and free-range poultry. We sampled 518 wild parrots and 247 free-range chickens in Victoria, Australia, and tested for bacterial presence using PCR, sequencing and serology. We found *Chlamydia* in both parrot and chicken samples. PCR analysis and sequencing of parrot samples indicated that *C. psittaci* prevalence was approximately 6.1% (95% confidence interval [CI] = 4.2, 8.5), and that the overall prevalence of order Chlamydiales bacteria was 23.4% (CI = 19.8, 27.4.). We identified *C. gallinacea* in at least one parrot sample. Adult parrot seroprevalence was 29% (Cl 22.3, 36.1), however seroprevalence varied significantly between parrot species. In chickens, C. psittaci PCR prevalence was 1.2% (CI 0.3, 3.5), and C. gallinacea PCR prevalence was 14.6% (Cl 10.4, 19.6). Our results suggest that there may be more *Chlamydia* species circulating in wild birds than previously reported, and we provide the first report, to our knowledge, of C. gallinacea infection in wildlife. Our findings are consistent with findings elsewhere that C. gallinacea is more prevalent in chickens than C. psittaci. Our work highlights the need for further study of Chlamydia and other Chlamydiales in both wild and domestic hosts, including the impacts different chlamydial species may have, and possible pathways of interspecific transmission.

# URBANIZATION PREDICTS INFECTION RISK BY A PROTOZOAN PARASITE IN NON-MIGRATORY POPULATIONS OF MONARCH BUTTERFLIES FROM THE SOUTHERN COASTAL U.S. AND HAWAII

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## Abstract

Urbanization can affect the density of hosts, altering patterns of infection risk in wildlife. Most studies examining associations between urbanization and host-parasite interactions have focused on vertebrate wildlife that carry zoonotic pathogens, and less is known about responses of other host taxa, including insects. Here we ask whether urban development predicts infection by a protozoan, Ophyrocystis elektroscirrha, in three populations of monarchs (Danaus plexippus): migratory monarchs in northeastern U.S., non-migratory monarchs in southeastern coastal U.S., and non-migratory monarchs in Hawaii. We used impervious surface and developed land cover classes from the National Land Cover Database to derive proportional measures of urban development and an index of land cover aggregation at two spatial scales. Parasite data were from previous field sampling (Hawaii) and a citizen science project focused on monarch infection in North America. Proportional measures of urban development predicted greater infection prevalence for non-migratory monarchs sampled in the southern coastal U.S. and Hawaii, but not in the northern U.S. Aggregations of low intensity development, dominated by single-family housing, predicted greater infection prevalence in monarchs from the northern and southern coastal U.S. populations, but predicted lower infection prevalence in Hawaii. Because natural habitats have been reduced by land-use change, plantings for monarchs in residential areas and urban gardens has become popular among the public. Mechanisms that underlie higher infection prevalence in urban landscapes remain unknown. Further monitoring and experimental studies are needed to inform strategies for habitat management to lower infection risk for monarchs.

#### BATRACHOCHYTRIUM SALAMANDRIVORANS IN THE CUBAN TREEFROG (OSTEOPILUS SEPTENTRIONALIS)

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### Abstract

Batrachochytrium salamandrivorans (Bsal) is a chytrid fungus that infects amphibians, causing skin lesions and eventually death. To date, it has been thought that Bsal only leads to asymptomatic carrier states in frog species and does not cause disease as it does in susceptible salamander species. Therefore, we hypothesized that anurans would not develop disease when challenged with concentrations of Bsal that result in disease in salamanders. To test this hypothesis, we exposed invasive Cuban treefrogs (Osteopilus septentrionalis) to either a mock inoculation of autoclaved dechlorinated water or one of four concentrations of Bsal zoospores 5x10<sup>3</sup>,  $5x10^4$ ,  $5x10^5$ , or  $5x10^6$  via water bath (n = 4 per zoospore dose). These concentrations were chosen based on our experience in testing 23 species of salamanders with varying ranges of susceptibility and successfully eliciting disease. Frogs were swabbed every six days during water-change events to document the onset and progression of infection. Frogs exposed to 5x10<sup>6</sup> zoospores tested positive for *Bsal* using qPCR starting four days post-exposure. Beginning two weeks post exposure, these frogs began showing signs consistent with Bsal chytridiomycosis, including darkened pigmentation on the dorsum, which progressed to red spots that appeared to be microhemorrhages. Infected animals also developed erythema and hemorrhage on the feet and ventral aspect of the abdomen, as well as excessive shedding on the feet. One frog in the  $5x10^6$  group was euthanized and two died naturally. Histopathology was performed on all three cases; multiple erosive crater-like epidermal lesions were randomly distributed throughout the body and varied from superficial to full thickness epidermis. Lesions consisted of focal epidermal necrosis with numerous intralesional Bsal thalli that were evident when stained with H&E. This challenge experiment represents the first documentation of *Bsal* chytridiomycosis in an anuran species and emphasizes that the conservation threat of *Bsal* may extend beyond salamander species.

#### PATHOLOGY IN SNAKES NATURALLY AND EXPERIMENTALLY INFECTED WITH OPHIDIOMYCES OPHIODIICOLA

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#### Abstract

**Background**: Ophidiomycosis (i.e., snake fungal disease) is an important disease of wild snakes in North America. The causative fungus, *Ophidiomyces ophiodiicola*, was first recognized as a cause of dermatitis and death in wild snakes in 2011. Developing a reliable experimental model is integral to furthering our understanding of ophidiomycosis in wild snakes.

**Objective**: Compare pathology and lesion distribution associated with ophidiomycosis in naturally-infected wild snakes and experimentally-infected cornsnakes (*Pantherophis guttatus*).

**Methods**: Gross and microscopic findings from wild snakes diagnosed with ophidiomycosis through histopathology and qPCR at the Canadian Wildlife Health Cooperative from 2012-2018 were reviewed and compared with the findings from twenty-three experimentally-infected juvenile cornsnakes that were subcutaneously injected with *O. ophiodiicola*.

**Results**: Twenty-one naturally-infected snakes (12 skin biopsies, 9 carcasses) had microscopic lesions consistent with ophidiomycosis and were positive via qPCR. Eighteen had consistent gross lesions in the skin. In 3/9 carcasses examined, ophidiomycosis was considered the cause of death. None of the cases had embolic fungal granulomas.

Almost half (11/23) of the experimentally-infected snakes developed gross skin lesions; 20/23 developed microscopic skin lesions at, and 2/23 distant to, the inoculation site. The majority (21/23) also developed systemic infections, including fungal granulomas in liver, coelomic connective tissue, thymus, and lung. All snakes developed microscopic lesions with visible fungus and were positive via qPCR. All livers with fungal granulomas tested qPCR positive.

Lesions were similar in experimentally- and naturally-infected snakes; gross lesions included crusting, ulceration and vesicle formation, and microscopically, heterophilic and granulomatous dermatitis, myositis, and osteomy-elitis were observed.

**Conclusion**: Pathological manifestations of ophidiomycosis were similar in naturally- and experimentally-infected snakes. However, subcutaneous inoculation with *O. ophiodiicola* in cornsnakes often caused systemic fungal infections, which was not observed in natural cases. Further surveillance and diagnostic evaluations are needed in wild snakes to assess for systemic infections.

# OUTBREAK OF SEVERE DERMATOMYCOSIS IN FREE-LIVING EASTERN WATER DRAGONS IN BRISBANE, AUSTRALIA ASSOCIATED WITH NANNIZZIOPSIS BARBATA-LIKE FUNGAL PATHOGEN

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#### Abstract

Emerging fungal diseases pose a significant threat to biodiversity and have driven declines and extinctions across a range of taxa. In recent decades there has been an increasing number of reports globally of fatal dermatomycoses in reptiles associated with fungi belonging to the genera Nannizziopsis, Paranannizziopsis and Ophidiomyces, formerly known as the Chrysosporium anamorph of Nannizziopsis vriesii (CANV) complex. These fungal pathogens cause severe skin lesions and systemic disease in a wide range of reptile species and are now recognized as significant emerging threats to reptiles worldwide. Infections caused by *Nannizziopsis* are often fatal, with limited treatment success. Until recently, reports worldwide have been restricted to captive animals. Starting in 2014, a small number of free-living Eastern water dragons (Intellagama lesueurii) in Southeast Queensland, Australia, were observed with severe dermal lesions. Since that time, we have noted a substantial increase in the number of Eastern water dragons presenting with similar skin lesions, and have now detected cases in multiple widely-separated locations across Brisbane, Queensland. To identify the cause of this outbreak, nine cases of severe disease from five locations were investigated using histology, culture and PCR. In all cases, histopathology of skin lesions revealed fungal invasion of the epidermis. Fungal culture from affected lesions yielded a fungus morphologically consistent with Nannizziopsis species. From fungal isolates, skin swabs and skin scrapings of affected lesions, PCR and genetic sequencing of the ITS region identified an organism with closest similarity to Nannizziopsis barbata, which has previously been associated with systemic, fatal disease in captive coastal bearded dragons (Pogona barbata) in Australia. Infection with Nannizziopsis is known to cause fatal disease in a wide range of reptiles, including species native to Australia. This outbreak, now confirmed in free-living Eastern water dragon populations across multiple locations, poses a serious threat to Australia's reptilian biodiversity.

# SPATIAL ANALYSIS OF HEMORRHAGIC DISEASE AND PHYSIOGRAPHIC REGION IN THE EASTERN UNITED STATES

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### Abstract

Hemorrhagic disease (HD) is considered one of the most significant infectious diseases of white-tailed deer in North America and the epidemiology of the disease has continued to evolve in the sixty plus years since the discovery of the causative agents. Investigations into environmental conditions associated with outbreaks suggest droughts are strongly correlated with outbreaks. However, during 2017 an HD outbreak occurred in the Eastern United States which appeared to be associated with a specific physiographic region, the Appalachian Plateau, and was not associated with any known drought conditions. The objective of this study was to determine if outbreaks of HD in white-tailed deer in 2017 were correlated with physiographic region. To assess this correlation, we performed basic summary statistics, logistic regression, and spatial analysis using a centered autologistic regression model to determine the significance of the relationship between physiographic regions and HD county-level reports from the Eastern United States. There were 456 reports of HD from 1605 counties across 26 states and 12 physiographic regions. Of the 93 HD reports confirmed by virus isolation, 76.3% (71/93) were identified as EHDV-2 and 66.2% (47/71) were from the Appalachian Plateau. A report of HD was 4.4 times more likely to occur in the Appalachian Plateau than not in 2017. Interestingly, Interior Low Plateau was also significantly correlated with a report of HD in 2017. The autologistic models suggested there was significant spatially dependence. Including virus isolation data enhanced our analysis and supported the claim that the outbreak in 2017 was strongly correlated with physiographic region. The underlying factors explaining this correlation are unknown, but may be related to a variety of host, vector, or environmental factors. This unique outbreak and its implications for HD epidemiology highlight the importance for increased surveillance and reporting efforts in the future.

# MAKING A MOUNTAIN OUT OF A RAT HOLE: COULD CURRENT RAT CONTROL PRACTICES HAVE UNINTENDED CONSEQUENCES?

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## Abstract

**Background**: Urban Norway rats (*Rattus norvegicus*) are the source of several pathogens transmissible to people. Transmission of these pathogens among rats may be determined by rat social structures, suggesting that interventions (e.g., pest control) that disturb stable colonies could affect pathogen ecology. The objective of this study was to evaluate how a simulated pest control intervention could impact the carriage of three bacterial zoonoses in rats: *Leptospira interrogans* (transmitted through contact with urine), methicillin-resistant *Staphylococcus aureus* (MRSA) (transmitted through direct and indirect contact, e.g., environmental fecal contamination), and *Bartonella* spp. (transmitted by rat fleas).

**Methods**: The study area included 36 blocks in Vancouver, Canada, which were subdivided into 12 groups of 3 blocks. In 5 of these block groups, we enacted a kill-trapping intervention in the central block; the other 8 block groups were controls. Rats (N = 513) were trapped using capture-mark-recapture for 2 weeks before and after a 2-week intervention. We collected urine (tested for *L. interrogans* using PCR), fecal/oral swabs (tested for MRSA using culture), and blood and fleas (tested for *Bartonella* spp. using PCR) for each rat. Multi-level logistic regression was used to identify the impact of the intervention on pathogen carriage while controlling for confounding variables (e.g., mass, sex, bite wounds).

**Results**: Rats caught in the intervention blocks following an intervention had a greater odds of carrying *L. interrogans* (OR: 9.55; 95% CI: 1.75–78.31), than did rats caught before the intervention. There was no impact on pathogen carriage in the surrounding blocks, and there was no impact on carriage of MRSA. Analysis of *Bartonella* spp. prevalence is ongoing.

**Conclusions**: These results suggest that current, lethal rat control techniques can have unintended and paradoxical effects on pathogen prevalence, which could increase human health risks. However, these effects depend on the mode of transmission of the pathogen.

#### PATHOGENS OF ZOONOTIC AND CONSERVATION CONCERN IN LOS ANGELES COUNTY, CALIFORNIA

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## Abstract

Many pathogens carried by urban wildlife can be transmitted to humans and domestic pets, but much remains unknown about the abundance, population dynamics and pathogen loads of the urban mammals inhabiting many large metropolises. To address these existing knowledge gaps in Los Angeles County, we are finalizing a 3-year pathogen survey of five common mammal species (coyotes, raccoons, skunks, opossums and squirrels) that may carry zoonoses (e.g. Leptospira, Giardia and Cryptosporidium spp.) and pathogens of conservation concern (e.g. canine distemper virus – CDV). To assess fecal pathogen infections in raccoons and coyotes, we analyzed feces (n=87) for pathogen DNA using polymerase chain reaction (PCR), with positive results for CDV (n=50), Salmonella (n=11), Cryptosporidium (n=2) and Giardia (n=2). In all species, we assessed prior Leptospira exposure via microscopic agglutination testing (n=186) and active infections using Leptospira PCR (n=207). Leptospira exposure was detected in coyotes (23%, n=35), Northern raccoons (36%, n=50), fox squirrels (41%, n=44), striped skunks (23%, n=13) and one Virginia opossum (2%, n=44). We detected active *Leptospira* infections in coyotes (4%, n=53), Northern raccoons (5%, n=20), striped skunks (13%, n=15) and Virginia opossums (2%, n=51), but not in fox squirrels (n=68). By mapping pathogen occurrence in relation to indices of urbanization, we gain a qualitative understanding of factors governing pathogen distribution across this urban landscape. Different patterns of Leptospira serovar reactivity in different species also indicate that there are likely multiple strains of this bacteria circulating in the Los Angeles area. This study provides an initial assessment of pathogens of zoonotic and conservation concern in L.A. County mammals across an urbanization gradient. Pathogen results will additionally be paired with host genetics to increase our knowledge of urban mammal connectivity, which together will inform local wildlife, veterinary and public health agencies about pathogen occurrence and transmission potential in these urban mammals.

#### SEASONAL SHEDDING OF CORONAVIRUSES IN URBAN COLONIES OF STRAW-COLORED FRUIT BATS

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## Abstract

Bats play important ecological roles; however, they have also been proposed as the natural host of several zoonotic viruses, such as the Severe Acute Respiratory Syndrome virus. Due to anthropogenic environmental changes several bat species have adapted to urban environments creating intense "bat-human" interfaces. Understanding viral dynamics in these urban-adapted populations can lead to data-driven mitigation strategies aiming to reduce spillover risk if zoonotic viruses are shed by the bats whilst supporting a healthy human-bat coexistence. The straw-colored fruit bat (*Eidolon helvum*) is a common macrochiropteran that roosts in busy urban centers across Sub-Saharan Africa. These roosts vary seasonally in size, from tens of thousands to over a million individuals. We hypothesize that colony size impacts viral shedding with larger colonies presenting higher contact rates, leading to variable infection and increased shedding at certain times of the year. We also hypothesize that the annual birth pulse could result in increased shedding as well due to the increment of susceptible individuals. To assess these hypotheses, we studied two E. helvum urban roosts in Tanzania and Ghana. We collected 97 fecal samples per month per roost over a one year period (n = 2,328) and tested them by consensus PCR for coronaviruses. In parallel, we censused these colonies and documented the presence of newborns. Statistical analysis revealed that coronavirus shedding does have a seasonal pattern, which may be driven by colony size and annual birth pulse. Our results provide evidence to support mitigation strategies to protect public health during in specific periods should these bats shed known zoonotic viruses. Under this scenario, avoiding roosts and reducing contact with bats during high-risk periods may help prevent spillover, while encouraging bat-human coexistence.

# CLIMATIC SUITABILITY OF SAN JOAQUIN KIT FOX (VULPES MACROTIS MUTICA) DENS FOR SARCOPTIC MANGE (SARCOPTES SCABIEI) TRANSMISSION

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#### Abstract

Over 460 cases of sarcoptic mange (Sarcoptes scabiei) in endangered, California endemic San Joaquin kit foxes (Vulpes macrotis mutica, SJKF) has been reported and is decimating the historically stable population in Bakersfield. This is a contact-driven disease and this fox has little intra-species contact outside of family groups. As SJKFs are a den-obligate species, their dens have been proposed as a second route of transmission allowing propagation despite medical interventions. In this study, it the SJKF den temperatures and humidities were determined and assessed for their ability to support mite off-host survival for at least 2-3 days. SJKF dens were monitored for 6 days each using temperature- and humidity-sensing iButtons placed within the dens. Motion-triggered cameras monitored animal use of the dens. A linear regression model was fitted to data from Arlian et. al 1989 to predict estimated mite survival time (EMST) in SJKF dens with observed den mean temperature and humidity. Den covariates including irrigation, type of den, and season were fitted to a mixed effects linear model to predict EMST. The average EMST across all measured dens was 4.8 days. Covariates of season (summer, or winter) and den type (grass, manmade or earth dens) were found to significantly (p<0.05) add to the model providing the longest EMST of 7.1 days for dens in habitats with irrigated grass in the winter. This supports that den climatic conditions in Bakersfield, California may support off-host mite survival through a timeframe adequate for re-visitation by another fox. The finding that grass dens may enhance EMST provides a valuable tool for SJKF management to mitigate this epidemic of mange. Treatment of the dens with forced heated air and building of artificial dens to discourage use of grass dens could be added to the current medical management to improve control efforts of this epidemic.

# MOLECULAR EPIDEMIOLOGY OF A FATAL SARCOPTIC MANGE EPIDEMIC IN ENDANGERED SAN JOAQUIN KIT FOXES (VULPES MACROTIS MUTICA)

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#### Abstract

Sarcoptic mange, caused by the Sarcoptes scabiei mite, was associated with a catastrophic population decline beginning in 2013 in a formerly stable urban population of endangered San Joaquín kit foxes (Vulpes macrotis *mutica*) in Bakersfield, California. To determine whether sympatric canids share manage mites and whether management effort to control the epidemic must involve not only kit foxes but also dogs (*Canis lupus familiaris*), coyotes (Canis latrans), and red foxes (Vulpes vulpes), we evaluated genotypes of mites collected from each host species and gene flow among them. We used 10 Sarcoptes microsatellite markers (SARM) to perform molecular typing of 351 S. scabiei mites collected from skin scrapings from five infested kit foxes, two dogs, five coyotes, and five red foxes from Bakersfield and nearby cities in California. We identified 61 alleles across all SARM loci and found distinct mite populations unique to particular hosts. There was a strong heterozygote deficit and most loci were not in Hardy-Weinberg Equilibrium. Kit fox-and red fox- derived mites were the most monomorphic (70%), while genetic variability was greatest in coyote- and dog-derived mites. Statistically significant linkage disequilibrium was detected in coyote- and dog-derived mites across most SARM loci, while SARM-33 and SARM-40 appeared linked in kit foxes and Bakersfield red foxes. AMOVA analysis showed documented the differentiation among the mite-derived hosts with an overall  $F_{s_T}$  of 0.465. The lowest  $F_{s_T}$  (i.e. closest genetic relationship,  $F_{st}$  = 0.05) was between kit fox- and red fox-derived mites while the largest genetic difference was between kit fox- and dog-derived mites (FST = 0.70). These results demonstrate some Sarcoptes host specificity, suggesting that, although an inter-species spillover event likely initiated the kit fox mange outbreak, mite transmission is now primarily intraspecific. Therefore, any large-scale population level intervention should focus on treating foxes within the city.
# A RETROSPECTIVE STUDY ON ADMISSION TRENDS OF HUMMINGBIRDS FROM CALIFORNIA-BASED REHABILITATION CENTERS OVER A PERIOD OF 26 YEARS (1991-2016)

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# Abstract

Hummingbirds are one of the most common taxa of wildlife presented to Californian rehabilitation centers, accounting for approximately 5% of admissions. Hence, identifying factors related to their age, sex, and reason for presentation could impact survivability and help shape efforts to maximize rehabilitation. Mixed effects logistic regression models were used to identify specific threats to the survival of 6,931 urban hummingbirds (1,618 nestlings, 5,313 non-nestlings), constituting of six species, admitted to wildlife rehabilitation centers in California over the past 26 years. In total, 36.05% birds survived to the point of being able to be transferred to flight cage facilities for further rehabilitation and/or release. Nestlings were more likely to be transferred compared to other age groups (survival odds ratio 4.13, p<0.005). After accounting for age, seasons were found to be significantly affecting the survival, with least mortality in spring (high survival odds ratio 2.76, p<0.005). Highest number of nestlings were rescued during the spring which coincides with the nesting season of hummingbirds in California with least number of rescues in fall. Reasons for presentation to rehabilitation centers included several anthropogenic factors such as window collisions (9.3 %) and interaction with companion pet animals (12.7 %). Hummingbirds were less likely to survive after being rescued in a torpor-like state (survival odds ratio 0.45, p<0.005). Preliminary data analysis for hummingbirds medically treated at wildlife centers identified primary treatment options, including providing sugar water (odds ratio 2.07, p<0.005), that significantly influenced hummingbird survivability at rehabilitation centers. Our results provide evidence of key issues for urban hummingbirds and suggest potential mitigation of anthropogenic threats.

## CHLAMYDIAL INFECTIONS IN BIRDS OF PREY PRESENTED TO REHABILITATION FACILITIES IN CALIFORNIA

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# Abstract

Chlamydial infections, caused by a group of obligate intracellular gram negative bacteria, have health implications for birds and humans. Due to their highly infectious nature and zoonotic potential, staff at raptor rehabilitation centers should be educated on the prevalence, types, and clinical presentations of *Chlamydia* spp. infections to prevent transmission to other birds and humans. The objectives of this study were to capture signalment, physical examination findings, and Chlamydial quantitative PCR (rt-qPCR) and IgM/IgY serological testing for raptors admitted to five rehabilitation centers in California (CA) over a 1-year period. We estimated Chlamydial prevalence in raptors for each center and evaluated potential risk factors for infection, including location, species, season, sex, and age class. Plasma samples and swabs from the conjunctiva, choana, and cloaca were collected for rt-qPCR from 263 birds of prey representing 18 species. Swab samples were sequenced via rt-qPCR based on the ompA gene and representative samples were compared with all known C. psittaci strains (A-G, E/B, M56 and WC) and *C. pneumoniae*. Aliquots of the plasma were sent to commercial laboratories for IgM testing via elementary body agglutination (n=190) and IgY using indirect fluorescent antibody testing (n=186). The overall prevalence by rt-qPCR was 4.18% (11/263) and seroprevalence was 3.14% (6/191). Three of the positive rt-qPCR birds (one Swainson's hawk (Buteo swainsoni) and two red-tailed hawks (Buteo jamaicensis)) were necropsied, and tissues were collected for culture and whole genome sequencing. Sequencing confirmed infection by a Chlamydial organism that is most similar to but distinct from C. psittaci genotype M56 and *C. abortus*. A spatial cluster of seropositive raptors and raptors testing positive for *Chlamydial* spp. DNA by rt-rt-qPCR was detected in northern California between Sacramento and San Francisco. These results describe a potentially new Chlamydial species infecting birds of prey in CA.

# EARLY DETECTION OF HEALTH EVENTS THROUGH TEMPORAL ANOMALIES IN WILDLIFE ADMISSIONS AT REHABILITATION CENTERS

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# Abstract

Wildlife rehabilitation centers enhance detection of known and emerging health threats through real-time integration of disease-specific and pre-diagnostic data. We developed new data-informed thresholds based on pre-diagnostic clinical classifications (e.g., neurological disease) to detect anomalous wildlife health events for the "Wildlife Morbidity and Mortality Event Alert System" (WMMEAS), which runs in parallel with the Wildlife Rehabilitation MD (WRMD), a free on-line database developed in 2014. The WMMEAS integrates data entered near real-time by centers across California and uses baseline thresholds to identify unusual morbidity and mortality events. Baseline thresholds were defined from statewide admissions data. Here we show how we improved upon them by developing thresholds based on 4 years of WRMD data that account for seasonality and trends in admissions. Using natural language processing, we classified over 200,000 patient records into twelve clinical classifications. Admissions among taxonomic families and species, and taxa-clinical classification combinations were evaluated for temporal anomalies. An anomalous event was triggered when the number of admissions exceeded two standard deviations above the rolling mean. Using new thresholds, we conducted time-series analyses to assess trends in admissions of columbids with neurological disease and explore whether anomalous patterns corresponded to disease outbreaks recognized by the state wildlife agency. The model correctly categorized cases into clinical classifications with high positive predictive values (range 0.73-0.99). Compared to the baseline thresholds, the new thresholds were able to account for seasonality and automatically generate alerts specific to clinical classifications. The new thresholds identified anomalies for neurological disease in Columba livia and Streptopelia decaocta in April and August of 2017, respectively. Investigations confirmed PPMV-1 and Sarcosystis calchasi in Streptopelia and Columba species, respectively. These coincided with the emergence of PPMV-1 in northern California and an outbreak of Sacrocystis calchasi. Results highlight the utility of data-driven thresholds for enhanced detection of unusual health events.

#### DISEASE RISK ANALYSIS FOR RE-INTRODUCTION OF WOLVES TO ISLE ROYALE NATIONAL PARK, USA

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## Abstract

Consideration of disease risks are becoming more common in wildlife translocations and re-introduction projects due to increasing awareness of consequences for species conservation, wildlife health and human health. Frameworks for disease risk analysis have been developed, but examples of how these tools can be applied when planning for wildlife translocations are limited. We integrated a disease risk analysis into planning for re-introduction of wolves to Isle Royale National Park, a project that was initiated in 2018 to restore predation, a key ecosystem community dynamic on the island. We used the IUCN/OIE Guidelines for Disease Risk Analysis (DRA) to qualitatively assess and prioritize disease risks for wildlife and human health associated with capture and translocation of wolves. Outcomes of the DRA were used to determine mitigation measures including: 1) criteria for selection of wolves for translocation, 2) disease surveillance at time of capture, 3) targeted treatments and 4) biosecurity measures to prevent disease transmission. Results from disease surveillance and post-release monitoring will be used to reassess and refine the DRA. This iterative process allows us to adapt in response to new information or changing situations throughout the duration of the project.

# HIGH PREVALENCE OF HEART ANOMALIES OF SUSPECTED GENETIC ORIGIN IN A REINTRODUCED EURASIAN LYNX (LYNX LYNX) POPULATION, SWITZERLAND

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# Abstract

Lynx lynx was reintroduced to Switzerland in the 1970s, resulting in two populations (Jura, Alps) currently estimated to 200 independent individuals together. In 2003 an adult male Alpine lynx with a heart murmur died of a cardiomyopathy with severe histological heart lesions; a few more fatal cases and a high prevalence of similar subclinical heart lesions were found in the Alpine population, mainly in adult males. Heart murmurs were detected in an increasing number of live Alpine lynx. Genetic analyses revealed a reduced heterozygosity of Swiss lynx, particularly in the Alps. This study aimed at evaluating the clinical significance of heart murmurs in Swiss lynx and at assessing a potential genetic origin of cardiac anomalies. From 2000 to 2018, 202 heart auscultations were performed on 101 lynx (3 months-17 years of age); all were blood-sampled at capture. Serum samples from 71 lynx were available to measure two cardiac biomarkers: cardiac Troponin I (cTnT) and N-terminal pro-Btype natriuretic peptide (NT-proBNP). Analyses followed a case-control study design (with versus without murmur). Genetic analyses were performed using microsatellites; pedigrees were established with both genetic and field data. Nearly 50% of Alpine lynx had a heart murmur, mainly adult males; it was a rare finding in the Jura. Both biomarkers were significantly higher in the case than in the control group, with record values in the first lynx with fatal cardiomyopathy. First attempts to merge histopathological and clinical data with the lynx pedigree suggest an inheritance of the observed cardiac anomalies: affected animals were more closely related than expected from a random population sample. Our data suggest that (1) heart murmurs in lynx are associated with a heart disease; (2) a link exists between clinical and post-mortem findings; (3) this could indicate the start of inbreeding depression urging for genetic management of Eurasian lynx.

# HERPESVIRUS INFECTIONS IN LUMHOLTZ'S TREE-KANGAROO (DENDROLAGUS LUMHOLTZI): THE NATURAL AND THE UNNATURAL

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# Abstract

Our surveys of wild and captive Lumholtz's tree-kangaroo (*Dendrolagus lumholtzi*) detected, using PCR, a novel endemic gammaherpesvirus in healthy animals, as well as a fatal infection with a non-endemic alphaherpesvirus. A novel gammaherpesvirus was detected using PCR and DNA sequence confirmation in free-ranging animals, in wild-caught captive animals and in one captive bred individual. An alphaherpesvirus, macropodid alphaherpesvirus 4 (MaHV4), was detected by PCR and DNA sequence confirmation in tissues from a captive-born Lumholtz's tree-kangaroo that died after an acute illness. Serologic (virus neutralization) evidence of alphaherpesvirus infection (using macropodid alphaherpesviruses 1 and 2) was found only in captive tree-kangaroos with a possible association with clinical disease and sudden deaths. Captive animals were potentially exposed to other macropod species while in care through the common practice of co-housing difference species together, allowing intimate contact that would not occur in nature. Our findings highlight the potential threats from unnatural disease exposure, and raise concerns that the release of 'rescued' tree-kangaroos back into the wild may result in the introduction of a potentially fatal, non-endemic alphaherpesvirus into the naïve population of free-rang-ing Lumholtz's tree-kangaroo.

# ASSESSMENT OF MOJAVE DESERT TORTOISE HEALTH INSIDE AND OUTSIDE THE LARGE-SCALE TRANSLOCATION SITE

Tristan Burgess<sup>1</sup>, Josephine Braun<sup>2</sup>, Carmel Witte<sup>2</sup>, Nadine Lamberski<sup>2</sup>, Roy Averill-Murray<sup>3</sup>, Kimberleigh Field<sup>3</sup>, Linda Allison<sup>3</sup>, Bruce Rideout<sup>2</sup>

<sup>1</sup>Acadia Wildlife Services, <sup>2</sup>San Diego Zoo Global, <sup>3</sup>U.S. Fish and Wildlife Service

# Abstract

The desert tortoise (Gopherus agassizii), has suffered habitat loss and fragmentation due to human development. Many tortoises, including pets and animals removed from development sites, were released into a fenced Large-Scale Translocation Site (LSTS) in the Ivanpah Valley, SW Nevada. Upper respiratory tract disease (URTD), a health threat to desert tortoises, has been detected at LSTS. This study, part of a project evaluating risks and benefits of removing fencing around LSTS, aims to: i) estimate prevalence of URTD and Mycoplasma infection at LSTS and three nearby unfenced sites, ii) assess whether Mycoplasma infection status was associated with developing clinical signs of URTD, iii) determine whether such an association differed between LSTS and unfenced areas. A total of 421 tortoises were sampled in 2016 (T2), of which 196 were previously sampled between 2011 and 2014 (T1). Clinical signs were recorded at field health assessments. Individual infection status for Mycoplasma agassizii and M. testudineum was determined by qPCR and ELISA. Multiple logistic regression was used to estimate associations while controlling for other covariates. In 2016, LSTS had the highest prevalence of M. agassizii (25%), M. testudineum (3.0%), and URTD signs (18.9%). Controlling for other factors, presence of any clinical sign(s) was positively associated with *M. agassizii* infection (OR=7.7, p=0.001), but not with study site (p≥0.127). There was no association with *M. testudineum* status (p=0.360). An estimated 3.2% of tortoises converted from *M. agassizii*-negative to-positive between T1 and T2 and incidence was greater at LSTS (p=0.002). Conversion of *M. agassizii* status was associated with increased incidence of clinical signs at T2 (OR=11.1, p=0.018). While *M. agassizii* and URTD are present outside the LSTS, there is a possibility that incidence of *M*. agassizii infection and URTD would increase outside the LSTS if these populations were to reconnect. Population-level significance of mycoplasmosis remains unknown.

# CAPTIVE BREEDING OF GREATER SAGE GROUSE (CENTROCERCUS UROPHASIANUS) FOR REINTRODUCTION: CHALLENGES AND SUCCESSES 2014-2019

Sandie Black<sup>1</sup>, Douglas Whiteside<sup>2</sup>, Adriana Pastor<sup>1</sup>, Emma Vaasja<sup>1</sup>

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## Abstract

The iconic greater sage grouse has experienced significant population declines on the Canadian prairies (almost 90% over the past three decades), largely due to habitat loss related to agricultural and industrial activities such as the conversion of prairie to cropland, overgrazing, and oil exploration. The species is currently listed as "Endangered" by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) with a breeding population of less than 1,000 birds. Due to this catastrophic decline, national recommendations for species recovery included captive breeding for reintroduction, and this was initiated at the Calgary Zoo to produce birds for bolstering the wild population. From 2014-16, forty-six eggs from wild greater sage grouse hens were brought into captivity, incubated, and hatched to create a captive flock. Although initial hatchability of these wild eggs was greater than 90%, several causes of chick mortality impacted the program in the early years including ventricular impactions, bacterial infections, and trauma. A captive population of 15 breeding birds in the spring of 2017 produced 69 chicks the first year of reproduction. With 51 birds in the breeding flock in 2018, captive production increased to 132 chicks; however fertility and hatchability of eggs were lower than in previous years. Optimal breeding set-ups for this lekking species are being investigated to maximize production while minimizing stress and competition which can have significant health impacts. Causes of mortality in the captive bred chicks included visceral and pulmonary aspergillosis, congenital or developmental limb deformities, bacterial infections, trauma, and ventricular impactions. From September through October 2018, a total of 82 juveniles were transported to soft release pens at release sites in Alberta and Saskatchewan, and held up to four weeks for pre-conditioning. A total of 66 juveniles were successfully released to native habitat, the captive flock currently numbers 54 birds.

#### THE PAN-CANADIAN APPROACH TO WILDLIFE HEALTH

Craig Stephen

Canadian Wildlife Health Cooperative

# Abstract

In the Spring of 2018, all federal, provincial and territorial Ministers responsible for biodiversity and conservation approved a new Pan-Canadian Approach to Wildlife Health. The Approach presents a vision for wildlife health to protect the socioeconomic, cultural, and ecological value of healthy wildlife. The Approach addresses four challenges: (1) how to cross administrative and disciplinary boundaries; (2) rapidly changing social and environmental conditions requiring an adaptive approach; (3) providing assurances throughout the second biggest country in the world that human activities are not negatively affecting wildlife health, or that wildlife health is not a risk and (4) the increasing reliance on ad hoc programs without sustainable or predictable resource. The four strategic goals of the Approach are to; (i) strengthen Canada's capacity to identify and reduce wild animal health threats that put conservation, public health, and social opportunities at risk; (ii) implement and assess programs and policies to sustain healthy wild animals and the positive contributions they make to Canada by reducing disparities and differences in capacity and information across the country; (iii) strategies that improve anticipation of wildlife health policy and practice needs in the face of rapidly changing social and environmental conditions; and (iv) improve efficiency and effectiveness of public services by working together. The approach focusses on four pillars of activity to achieve its goals; (1) health intelligence; (2) wildlife health stewardship, (3) innovation and (4) integrated governance. This talk will describe the history and contents of the Approach and an update on its current state of implementation.

## HAIR ELEMENT PROFILE AS AN INDICATOR OF WILDLIFE POPULATION HEALTH AND DEMOGRAPHICS

Jesper Mosbacher<sup>1</sup>, Anders Michelsen<sup>2</sup>, Mikkel Stelvig<sup>3</sup>, Jean-Pierre Desforges<sup>4</sup>, Igor Eulaers<sup>4</sup>, Christian Sonne<sup>4</sup>, Rune Dietz<sup>4</sup>, Bjørn Munro Jenssen<sup>5</sup>, Tomassz Ciesielski<sup>5</sup>, Syverin Lierhagen<sup>5</sup>, Trond Peder Flaten<sup>5</sup>, Marie Rønne Aggerbeck<sup>4</sup>, Gaël Le Roux<sup>6</sup>, Sophia Hansson<sup>4</sup>, Mike Suitor<sup>7</sup>, Steeve Cote<sup>8</sup>, Stephane Lair<sup>9</sup>, Joelle Taillon<sup>10</sup>, Vincent Brodeur<sup>10</sup>, Niels Martin Schmidt<sup>4</sup>, Susan Kutz<sup>1</sup>

<sup>1</sup>University of Calgary, <sup>2</sup>University of Copenhagen, <sup>3</sup>Copenhagen Zoo, <sup>4</sup>Aarhus University, <sup>5</sup>Norwegian University of Science and Technology, <sup>6</sup>CNRS EcoLab, <sup>7</sup>Government of Yukon, <sup>8</sup>Université Laval, <sup>9</sup>Universite de Montreal, <sup>10</sup>Government of Quebec

#### Abstract

Monitoring population health and abundance is at the core of species conservation and management. However, for remote locations, this can be logistically and financially challenging. Instead, health indicators can be practical alternatives to monitor and predict population trends in years between population surveys as they are closely linked to population demographics. A key indicator of health is the trace element status of an individual and population. Essential trace elements affect the health, immunity, survival, and reproduction of wildlife. Deficits or imbalances in elements have been linked to broad scale population declines in wildlife. Knowledge of element profiles and their role in population dynamics is, therefore, critical for conservation and management strategies of wildlife populations. Here we use hair element profiles as an indicator of broad scale population health and demography. Hair is a promising matrix for assessing element status as it is easily sampled and serve as a chemically stable retrospective archive of the animal's element and nutritional status at the time of growth. We will present data from several muskox populations across the arctic, from Canada to Greenland. Hair samples were analyzed with inductively coupled plasma mass spectrometry, while additional information on the health of the animals were derived from serum, feces and morphometrics. We show that the profile of essential elements was associated with calf recruitment and adult mortality rates over a 18-year period in a high Arctic muskox population, with poor recruitment and elevated mortality coinciding with years of low hair concentrations. This suggests that hair element profiles constitute an attractive and practical tool in wildlife management for inferring population demography. We recommend routine and harmonised sampling, archiving, and analysis of hair could and should be an important component of wildlife conservation and management.

#### A HEALTH BASELINE FOR NARWHALS (MONODON MONOCEROS) FROM THE EASTERN CANADIAN ARCTIC

Sandie Black<sup>1</sup>, Jack Orr<sup>2</sup>, Matilde Tomaselli<sup>1</sup>, James Simonee<sup>3</sup>, Natasha Simonee<sup>4</sup>, Pádraig Duignan<sup>5</sup>

<sup>1</sup>University of Calgary, <sup>2</sup>Department of Fisheries and Oceans Canada, <sup>3</sup>Arctic Connexion, <sup>4</sup>Pond Inlet, <sup>5</sup>The Marine Mammal Center

#### Abstract

Healthy wildlife are critical for ecosystem health, a productive economy, and food safety and security in the Arctic. Rapid climate change in the Arctic is potentially troubling for ice-dependent marine mammals such as narwhal (Monodon monoceros), that are highly sensitive to changes in sea ice and the marine environment. Multiple anthropogenic stressors related to climate change are predicted to cause deleterious health effects for this species. Environmental toxins, industrial activities, harvest, loss of sea ice, disease, ice entrapments, predation, commercial shipping and competing fisheries all have the potential to negatively impact narwhal populations. As apex marine predators, narwhal rely on and are part of the resilience of the Arctic marine ecosystem, yet very little is known about individual and population health or how they may adapt to a changing environment. Collecting data on Arctic cetaceans is challenging: severe weather, darkness in winter, marine environment, and an extensive species range allow few opportunities to gather data and samples. Using a transdisciplinary scientific approach, narwhal health data have been collected from 2004-2018. Health indices will be reported from over 50 live free-ranging narwhals from the eastern Canadian Arctic gathered through physical exams conducted during handling, hematology and serum chemistry profiles and serology for selected pathogens. To date, these narwhal appear naïve to Cetacean morbillivirus and Influenza, and have varying levels of exposure to Toxoplasma and Brucella sp. With the assistance of Inuit collaborators, thorough necropsy examinations were carried out on thirteen traditionally harvested narwhals to investigate disease caused by pathogens, toxins and neoplasia. Findings of interest included aural and pulmonary parasitism, pulmonary, endocrine and intestinal neoplasia, sarcocystosis and other mild inflammatory changes in myocardium and skeletal muscle. These findings constitute the foundation of a health baseline for narwhals, against which future changes can be measured.

# LEPTOSPIROSIS IN ALASKAN CARIBOU (RANGIFER TARANDUS GRANTI) AND REINDEER (RANGIFER TARANDUS TARANDUS)

Cristina Hansen, Kendrick Hautala, Kelly Walker, Melissa Clark

University of Alaska Fairbanks

## Abstract

Leptospirosis is caused by any of more than 200 serovars of *Leptospira* species and is presumed to be the most common zoonotic disease worldwide. It is is likely under-reported due to lack of awareness and vague clinical signs. Leptospirosis is more common in tropical and temperate climates, but its distribution is likely to shift north as global temperatures rise. Little is known about leptospirosis in cervids or in the Arctic, and most published surveys report only antibody titers. We aimed to do a comprehensive survey of Alaskan *Rangifer* species (caribou and reindeer) for presence of Leptospira infection and past exposure. We collected blood, urine, and kidney samples from wild, captive, and domestic *Rangifer* throughout Alaska and performed microscopic agglutination tests on serum, and PCR on blood, urine, and kidney samples. Five of 41 kidney samples (12.2%); 1 of 43 urine samples (2.3%); and 0 of 149 blood samples were positive for *L. interrogans* DNA. Fifteen of 149 serum samples (10%) tested were positive for leptospiral antibodies (13 to serovar bratislava, 1 to canicola, and 1 to grippotyphosa). Leptospiral DNA is present in Alaskan *Rangifer* tissue samples, and we have documented seropositive animals. However, there is no correlation between seropositive and PCR positive samples, which agrees with the literature. To get a complete picture of Leptospirosis in Alaska and the circumpolar North, increased sampling efforts are needed, with a focus on collecting complete sample sets and performing comprehensive diagnostics.

# UNDERSTANDING HEALTH CHALLENGES AND DRIVERS FOR THE DECLINING DOLPHIN AND UNION CARIBOU

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<sup>1</sup>University of Calgary, <sup>2</sup>Government of Nunavut, <sup>3</sup>Swedish Museum of Natural History, <sup>4</sup>Toronto Zoo

# Abstract

The role of infectious diseases and trace mineral deficiencies in widespread population declines of migratory tundra caribou are poorly understood. Dolphin and Union (DU) caribou (Rangifer t. groenlandicus xpearyi) is a distinctive ecotype that ranges between Victoria Island and the adjacent Canadian Mainland. This population is undergoing a population decline similar to most of the barren-ground caribou in the Arctic and Subarctic Regions. Initial investigations on exposure to pathogens and stress levels in this herd (2015-2016) suggested higher exposure to Brucellaspp. and alpha-herpesvirus, and apparently higher hair cortisol levels than other caribou herds. Additionally, local knowledge gathered from one of the harvesting Inuit communities in 2014 indicated poor body condition and increased observations of sick animals, including swollen joints and testicles among other findings. Altogether, these results indicate that infectious disease may play a role in the decline of this herd, perhaps exacerbated by nutritional deficiencies. Our goal is to perform a comprehensive health assessment of DU caribou and evaluate the association of various infectious diseases and trace mineral profiles with individual health and reproduction. We obtained blood on filter paper (FP) and tissues from 165 animals sampled between 2015 and 2018 through an established hunter-based sampling program and collaring programs. Serology was used to determine exposure to prevalent infectious agents (Brucellaspp., Pestivirus, Neospora caninum, Toxoplasma gondii, Erysipelothrix rhusiopathiae and alpha-herpesvirus) and hair from the neck was used to determine trace mineral profiles and cortisol levels. The pregnancy status of all cows was determined through pregnancy-associated proteins in FP, and basic information such as body condition, sex and age were recorded from all the animals. This communication will present the relationship among all these parameters and survival and reproduction of the cows, in order to provide better knowledge of diseases in the declining process of DU caribou.

## DETECTION OF BARTONELLA SPP. IN CERVIDS BLOOD AND ECTOPARASITE POOLS FROM NORWAY

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<sup>1</sup>Laboratory of Wildlife Comparative Pathology - LAPCOM, School of Veterinary Medicine and Animal Sciences - FMVZ, University of São Paulo, <sup>2</sup>Norwegian Veterinary Institute

# Abstract

Bartonellosis might represent an underestimated threat to the health of humans, and domestic and wild animals. In Norway, *Bartonella* spp. have been detected in cervids, mainly within the distribution area of the arthropod vector deer ked (*Lipoptena cervi*). We used PCR to survey the prevalence of *Bartonella* in blood samples from 141 cervids outside the deer ked distribution area (moose [*Alces alces*, n=65], red deer [*Cervus elaphus*, n=41], and reindeer [*Rangifer tarandus*, n=35]), in 44 pool samples of sheep tick (*Ixodes ricinus*, 27 pools collected from 74 red deer and 17 from 45 moose) and in black flies (*Simulium* spp., 120 pools of 6710 specimens).

*Bartonella* DNA was amplified in moose blood samples (75.4%, 49/65)- 92.9% of the northern specimens and 70.6% of those from southern Norway, and in red deer (4.9%, 2/41). All reindeer were negative. Additionally, *Bartonella* was amplified in 2 of 17 tick pools collected from moose, although red deer tick pool samples were negative. *Bartonella* was also obtained from 3 of 120 black fly pool samples. Identical sequences of *Bartonella* were amplified in moose, red deer and ticks, highly similar to *B. bovis*, previously identified in cervids. The sequence obtained from black flies only presented 81.7% identity to the closest *Bartonella* spp., and could be part of the gut microbiome of the Simuliidae. Herein we demonstrated that *Bartonella* is present in cohorts of moose across Norway- the first data available from northern Norway. The high prevalence of *Bartonella* in wild reindeer could make this species an useful sentinel to future surveys regarding the expansion of this pathogen. This is the first report of *Bartonella* sp. in ticks from Fennoscandia, and the first of *Bartonella* in black flies worldwide.

# STEJNEGER'S BEAKED WHALE STRANDINGS IN ALASKA, 1995 – 2018: HISTORY, DISTRIBUTION, AND NECROPSY FINDINGS

Kathy Burek-Huntington¹, Kate Savage², Anna Bryan³, Pam Tuomi⁴, Raphaela Stimmelmayer⁵, Martha Delaney⁵, Marc Webber², Gay Sheffield<sup>®</sup>

<sup>1</sup>Alaska Veterinary Pathology Services, <sup>2</sup>NOAA/NMFS, <sup>3</sup>Alaska Department of Fish and Game, <sup>4</sup>Alaska SeaLife Center, <sup>5</sup>North Slope Borough, <sup>6</sup>University of ILlinois Zoological Pathology Program, <sup>7</sup>Untied States Fish and Wildlife Service, <sup>8</sup>Alaska Sea Grant

# Abstract

The Stejneger's beaked whale (Mesoplodon stejnegeri) is a deep diving cetacean species, with the center of their range being the waters off the western Aleutian Islands in Alaska. The first suspected Alaskan specimen was a single mandibular ramus found on the Copper River Delta in 1927 and up until 1994, only 41 cases have been described in the literature. This presentation discusses 34 additional Alaskan strandings which occurred between 1995 and 2018, half of which occurred since 2013. Each stranding response has resulted in data that reinforces existing knowledge and/or provides new insight into the life history of this enigmatic species. From these data we have documented a northern range extension; identified a strong bias for female strandings; analyzed stomach contents, identified parasites and determined Cause of Death (COD). Twenty-three animals were necropsied. Stomach contents included several squid species from two different families, Gonatidae and Cranchiidae. One stomach also contained an amphipod (Gammaridea) and an isopod (Tecticeps alascensis), and another contained a plastic cup lid. Seventeen of these animals necropsied occurred due to a mass stranding, and COD could not be determined (MS-CBD). Trauma was COD for 3 cases consistent with barotrauma, Infectious disease complicated by possible barotrauma occurred in 2 cases and 1 case was categorized as malnutrition. Many of the Steineger's strandings in Alaska have occurred in geographically isolated areas that greatly challenge the stranding response capabilities and complicate the ability to determine cause of death and collect life history samples. More efficient responses in remote areas using newer techniques and an expanded stranding network are needed in order to yield a better understanding of the causes of the increasingly frequent mass strandings, possible human caused impacts and causes of death.

# CO-DEVELOPING AND CO-MONITORING WILDLIFE HEALTH INDICATORS: BRIDGING LOCAL, TRADITIONAL AND SCIENTIFIC KNOWLEDGE TO IMPROVE WILDLIFE HEALTH SURVEILLANCE AND RESPONSE IN THE ARCTIC

Susan Kutz<sup>1</sup>, Stephanie Peacock<sup>1</sup>, Fabien Mavrot<sup>1</sup>, Matilde Tomaselli<sup>1</sup>, Alehandro Aleuy<sup>1</sup>, Juliette Di Francesco<sup>1</sup>, Xavier Fernandez Aguilar<sup>1</sup>, Andrea Hanke<sup>1</sup>, Naima Jutha<sup>1</sup>, Pratap Kafle<sup>1</sup>, Kugluktuk Angoniatit Association Calgary<sup>2</sup>, Lisa-Marie Leclerc<sup>3</sup>, Jesper Mosbacher<sup>1</sup>, Olokhaktomiut Hunters and Trappers Committee Calgary<sup>4</sup>

<sup>1</sup>University of Calgary, <sup>2</sup>Kugluktut, Nunavut, <sup>3</sup>Government of Nunavut, <sup>4</sup>Ulukhatok, Northwest Territories

#### Abstract

Wildlife are essential for resilient ecosystems, productive economies, Indigenous culture, and food safety and food security in the Arctic. Despite this importance, many northern species are increasingly threatened by rapid climate warming and the consequent environmental changes and emergence of infectious disease. Effective wildlife management in this changing landscape requires early detection of, and response to, changes in population health. Recent caribou and muskox declines in North America, together with emergence of zoonotic diseases in these species highlight the importance of having effective wildlife population health surveillance across the Arctic. However, surveillance is challenged by vast and remote areas, high operative costs, and limited logistical and human capacity in polar regions. Identifying reliable indicators of health, and establishing efficient mechanisms for tracking these indicators, is central to effective wildlife management in the Arctic.

The ancestral, ongoing, and intimate associations of Inuit and First Nations people with their natural environment have resulted in a deep understanding of natural processes; an understanding that is invaluable for contemporary wildlife management. We have are partnering with Indigenous communities in the Canadian North, bringing traditional, local and scientific knowledge together, to address knowledge gaps in wildlife health ecology. We are cooperatively identifying key indicators of caribou and muskox health that are feasible to track and that can inform pro-active management. Drawing on our experiences with our partner communities, we discuss how data on these indicators can and should be informed by three complementary approaches: targeted scientific studies, hunter-based sampling, and systematic documentation of local and traditional knowledge. Implementation of this multi-faceted process will ensure early detection of changes in population health earlier than conventional population monitoring methods and passive surveillance, thus allowing more timely adaptive responses.

#### IS THERE A DUE DILIGENCE STANDARD FOR WILDLIFE HEALTH SURVEILLANCE

Craig Stephen, Patrick Zimmer

Canadian Wildlife Health Cooperative

## Abstract

Despite growing demands for wildlife surveillance, there is little guidance on the necessary performance standards for a surveillance program. There are 3 approaches that could be used to develop surveillance performance expectations; (i) epidemiological standards; (ii) conformation with national and international requirements and (iii) due diligence. Logistic, biological and fiscal constraints complicate direct application of epidemiological and national or international standards for surveillance in people or domestic animals to free-ranging wildlife. We reviewed Canadian literature and legislation and used key informant interviews to determine if a wildlife surveillance due diligence standard existed. Key informants cited due diligence to justify investment in wildlife health surveillance to satisfy trading partners and other animal health stakeholders, but a due diligence threshold could not be found in regulations, international obligations or the literature. The lack of a due diligence standard puts wildlife health surveillance managers at a disadvantage when trying to show public return on investment or when assessing the adequacy of surveillance efforts. The Canadian Wildlife Health Cooperative is undertaking the development of some of its own performance standards to meet the performance needs of the Pan-Canadian Approach to Wildlife Health. This presentation will introduce these standards and describe the plan for a 5-year assessment to establish quantitative or qualitative thresholds for acceptable performance. This assessment will include determining current performance levels and sharing these with stakeholders and funders to ascertain their fit for need.

#### FRESHWATER MUSSEL DIE-OFF RESPONSE DEVELOPMENT AND ONE HEALTH ASSESSMENT

Nancy Boedeker, Brant Fisher

Indiana Department of Natural Resources, Division of Fish and Wildlife

## Abstract

Freshwater mussels are among North America's most imperiled species. Die-offs are increasingly recognized as population threats, with etiologies frequently undetermined. Minimal health and disease data exists for freshwater mussels. Detailed plans and descriptions of techniques for thorough and rapid diagnostics to guide a targeted die-off response are lacking. This project's objectives are to develop die-off response protocols in coordination with partners nationwide and establish and compare baseline health parameters for freshwater mollusks in Indiana waterways. Study species include native Fatmucket (Lampsilis siliquoidea) and Plain Pocketbook (Lampsilis cardium) and non-native Asian clam (Corbicula fluminea), all common in Indiana. Methods involve: 1) collection of mollusks (20 per species per site) from three Wildcat Creek drainage sites under assessment for mussel translocation suitability, 2) determination of microbial populations (viral, bacterial, parasitic, and fungal) and antibiotic resistance of bacteria cultured, and 3) assay of hemolymph and tissue samples to determine analyte levels (including glycogen, stable isotopes, contaminants) and histologic tissue evaluation. Analysis of results, compared between species and sites and to water quality parameters, identifies potential pathogens associated with Asian clam that might threaten native species, evaluates potential interspecies competition based on dietary composition comparison, adds to assessment of the suitability of the three sites for translocations, and increases understanding of antimicrobial resistance in aquatic environments. Using common species, this study allows for optimization of techniques and protocols for use in diagnostic response to die-offs of potentially endangered species. It establishes baseline health parameters of multiple species at varied sites which is critical for interpretation of results in the event of a die-off. We seek partnerships for future expansion of this pilot study, incorporating additional locations and species over time, to generate data to develop risk mitigation strategies for microbes and contaminants that contribute to freshwater mussel morbidity and mortality.

# CHARACTERIZING THE WELLNESS OF WILDLIFE: A COMPREHENSIVE ASSESSMENT OF HEALTH, PATHOGEN PRESENCE, IMMUNE FUNCTION, AND POPULATION VIABILITY IN EASTERN BOX TURTLES (TERRAPENE CAROLINA CAROLINA)

Laura Adamovicz<sup>1</sup>, Sarah Baker<sup>2</sup>, Mark Merchant<sup>3</sup>, Matthew Allender<sup>1</sup>

<sup>1</sup>Wildlife Epidemiology Lab, University of Illinois College of Veterinary Medicine, <sup>2</sup>Arizona Game and Fish Department, <sup>3</sup>College of Science: Department of Chemistry, McNeese State University

# Abstract

Conservation strategies that support the wellness of wildlife are increasingly important for effective population management. Habitat degradation, a leading driver of ecosystem diseases, can negatively impact wildlife health; however, this is infrequently demonstrated in terrestrial reptiles. The objectives of this study were to comprehensively characterize individual and population health in wild eastern box turtles (*Terrapene carolina carolina*) from habitats of different quality. We hypothesized that turtles from poor quality habitats would have higher pathogen burdens, decreased innate immune function, and smaller, less stable populations. Turtles (N=507) from five sites were evaluated using physical examination (PE), hematology, plasma biochemistry, and protein electrophoresis from 2016-2018. Turtles were screened for four ranaviruses, three Mycoplasma spp., three herpesviruses, and *Terrapene* adenovirus using qPCR. Multivariable linear models were used to evaluate site-based differences in health parameters while controlling for demographic and temporal variables. In 2018, plasma from each population was assayed for bacterial killing capacity (BKC). Population viability analysis was performed to model population stability and sensitivity to female turtle loss. Turtles from the smallest population with the worst habitat quality (KSP) had higher total solids, heterophils, eosinophils, and bile acids and lower lymphocytes and BKC compared to turtles from the largest population with the best habitat quality (Forbes) (P < 0.05). The Forbes population was robust to annual loss of 1-3 adult female turtles, with a low probability of extinction (< 25%). In contrast, the KSP population was projected to go extinct in 100% of simulations with the annual loss of one female, indicating poor population stability and high susceptibility to stochastic events such as disease. These findings reflect improved health status, immune function, and population resiliency at Forbes; the site with the most favorable habitat. Appropriate habitat management strategies should be applied to promote wildlife health and support conservation goals.

# DEVELOPMENT OF A KILLER WHALE HEALTH DATABASE TO ASSESS INDIVIDUAL AND POPULATION HEALTH OF SOUTHERN RESIDENT KILLER WHALES (ORCINUS ORCA)

Forrest Gomez<sup>1</sup>, Betsy Lutmerding<sup>1</sup>, Lisa Clowers<sup>1</sup>, Risa Daniels<sup>1</sup>, Cynthia Smith<sup>1</sup>, Lynne Barre<sup>2</sup>, Kirsten Gilardi<sup>3</sup>, Stephanie Venn-Watson<sup>4</sup>, Joseph Gaydos<sup>3</sup>

<sup>1</sup>National Marine Mammal Foundation, <sup>2</sup>NOAA/NMFS, <sup>3</sup>Wildlife Health Center, School of Veterinary Medicine, University of California at Davis, <sup>4</sup>Epitracker

#### Abstract

Health databases are invaluable tools for assessing both individuals and groups. They have contributed to species management changes and in some cases, population growth. The southern resident killer whale (Orcinus orca, SRKW) population is an extensively studied endangered sub-population of fish-eating killer whales in the Northeastern Pacific Ocean. The population was listed as endangered in Canada and the United States in 2001 and 2005, respectively. Population numbers have not recovered and continue to decline. Currently there are only 74 SRKWs. Lack of prey and exposure to toxins, contaminants, boat traffic, and noise have been cited as key threats to the population and substantial efforts have been made to address them. UC Davis and the National Marine Mammal Foundation have collaborated to develop a comprehensive killer whale health database. This electronic medical records system permits data to be queried for individuals, within groups, and across populations to track individual animal health and better understand population declines and aid in population recovery. Currently, the health database includes individual identification, health assessment findings, photographs, field observations, biological sampling, contaminants, photogrammetry, sample archive information and necropsy findings. By partnering with multiple entities in the United States and Canada, the growing database also includes information from other killer whale populations. With continued investment, it will serve as a centralized, cloud-based, secure database that promotes collaboration between researchers, veterinarians, government agencies and non-profit organizations.

# FOOT-AND-MOUTH-DISEASE EPIDEMIOLOGY IN A HERD OF ITS NATURAL RESERVOIR HOST, AFRICAN BUFFALO (SYNCERUS CAFFER)

Brian Dugovich<sup>1</sup>, Bryan Charleston<sup>2</sup>, Francois Maree<sup>3</sup>, Eva Perez-Martin<sup>2</sup>, Katherine Scott<sup>3</sup>, Brianna Beechler<sup>1</sup>, Anna Jolles<sup>1</sup>

<sup>1</sup>Oregon State University, <sup>2</sup>The Pirbright Institute, <sup>3</sup>Agricultural Research Council

# Abstract

Foot-and-mouth disease virus (FMDV) is endemic in African buffalo (Syncerus caffer) in Southern Africa and has spillover potential to cattle on the wildlife-livestock interface causing region-wide socioeconomic hardships. FMDV is highly infectious, and most buffalo have been exposed by 2 years of age. Newly infected calves and carrier buffalo are the likely host individuals to be involved in FMDV transmission, which may put cattle at risk. In Kruger National Park, South Africa, we aimed to determine when the carrier state occurred and which adult buffalo were more likely to be carriers. We captured a herd of 60-70 buffalo in a 900ha enclosure within the park every 2-3 months for 3 years. Using FMDV PCR, virus isolation, and serology assays, we identified the infection classes of each buffalo at each time point: maternally-protected, susceptible, infected, carrier, and recovered. In adult buffalo, exposure to infected calves increased the carrier state (p = 0.002). We found that adult carrier animals were more likely to have a lower body condition score (p = 0.029) and were younger (p < 0.001) and male (p = 0.049). We also looked at reports of buffalo sighted outside of the park, compiled by the State Veterinary Service, to determine when buffalo were most likely to contact cattle. We expected the wet season to have the most stray buffalo due to fence damage from flooding. However, stray events were more likely in the dry season (p = 0.009). According to the demographic patterns we observed, thin and young male buffalo may pose the highest risk for FMDV transmission to livestock. Basic epidemiological information describing disease transmission on an individual-level can help manage spillover risk in the face of variable environments and shrinking conservation budgets.

# DEVELOPMENT OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION METHOD FOR RAPID DETECTION OF RESPIRATORY PATHOGENS IN BIGHORN SHEEP

Connie Brewster<sup>1</sup>, Karen Fox<sup>2</sup>, Jessica Jennings-Gaines<sup>3</sup>, William Edwards<sup>3</sup>, Halcyon Killion<sup>3</sup>, Mary Wood<sup>3</sup>, Sandra Quackenbush<sup>1</sup>

<sup>1</sup>Colorado State University, <sup>2</sup>Colorado Parks and Wildlife, <sup>3</sup>Wyoming Game and Fish Department

# Abstract

Bighorn sheep respiratory disease is a limiting factor for population growth in many bighorn herds in western North America, and failed recruitment is often attributed to lamb pneumonia. In Colorado, one proposed strategy for management of disease is to selectively remove adults with chronic, persistent upper respiratory infections and thereby decrease the transmission of pathogens from adults to lambs. Our objective was to develop rapid field assays that could be used to identify respiratory pathogens (leukotoxigenic *Pasteurellaceae*, Mycoplasma ovipneumoniae, and Pasteurella multocida) in nasal swabs. We developed loop-mediated isothermal amplification (LAMP) PCR tests for this purpose, although only tests for M. ovipneumoniae and P. multocida were adequate for field application. These assays require minimal equipment, minimal processing, and rapid results based on visual detection of a pink-to-yellow color change. The LAMP tests were applied to nasal swabs from a small (n=10) captive herd of bighorns with known chronic upper respiratory disease. Results were compared to standard methods of culture enrichment followed by PCR. The LAMP test identified *M. ovipneumoniae* in 3/10 (30%) of nasal swabs versus 1/10 (10%) of swabs tested by culture enrichment and PCR. The LAMP test identified P. multocida in 6/10 (60%) of nasal swabs versus 8/10 (80%) of swabs tested by culture enrichment and PCR. Preliminary application to a wild herd of bighorns with known chronic respiratory disease suggests that the *P. multocida* LAMP test may be predictive of presence/absence of sinus tumors in this setting. These tests may be useful for bighorn respiratory disease surveillance and management applications that require rapid results in the field.

# VALIDATION OF A FIELD-PORTABLE, HAND-HELD QPCR SYSTEM FOR DETECTING PSEUDOGYMNOASCUS DESTRUCTANS, THE CAUSATIVE AGENT OF WHITE-NOSE SYNDROME IN BATS

Kevin Keel¹, Sybil Amelon², Greg Turner³, Tracy Trazenovich⁴, Maria Chacon-Heszele⁵, John Huckabee⁶, Jean Leonhardt⁶, Jesse van Westrienen⁵, Max Perelman⁵, Patricia Thompson², Katie Haman

<sup>1</sup>Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, <sup>2</sup>US Forest Service, <sup>3</sup>Pennsylvania Game Commission, <sup>4</sup>UC Davis, <sup>5</sup>Biomeme Inc, <sup>6</sup>PAWS Wildlife Center, <sup>7</sup>Washington Department of Fish and Wildlife

# Abstract

White-nose syndrome (WNS), caused by the cold-loving fungus *Pseudogymnoascus destructans* (Pd), has decimated bat populations across North America. In some species and hibernacula mortality rates are greater than 95%. Since the discovery of Pd in North American bats in 2006, surveillance for this disease has focused primarily on screening hibernating bats using a series of diagnostic tools. However, the recent discovery of WNS in Washington highlights the need for a modified approach to allow for early detection of Pd in new areas. Such an approach must address differences in bat habits and bat habitats. Specifically, the lack of large aggregations of hibernating bats in the American Pacific Northwest necessitate a different approach to surveillance than monitoring hibernacula. The ability to rapidly diagnose Pd in bats using an assay that can be used in the field, would improve our ability to respond to and manage Pd. We evaluated the feasibility of using a hand-held field portable quantitative PCR (qPCR) system (Biomeme two3) for detection of Pd. We compare the results from a bench-top molecular approach, used by labs conducting WNS and Pd surveillance, to the Biomeme qPCR (two3) platform in a lab setting and in field trials. We were able to successfully implement the PCR in the field and the comparative results will be presented. The establishment and validation of a rapid, field assessable detection platform for Pd will significantly improve not only surveillance capacity, but also on-the-ground management and response efforts (i.e. site management, bat rehabilitation, decontamination and biosecurity at field sites, etc). This will further strengthen the conservation responses to protect bats across North America from the devastating impacts of WNS.

# WILDLIFE MORBIDITY AND MORTALITY EVENT ALERT SYSTEM: INTEGRATING WILDLIFE REHABILITATION CENTER DATA FOR EARLY AND ENHANCED DETECTION OF THREATS TO WILDLIFE HEALTH AND SURVIVAL

Terra Kelly<sup>1</sup>, Nicole Carion<sup>2</sup>, Krysta Rogers<sup>2</sup>, Stella McMillin<sup>2</sup>, Deana Clifford<sup>3</sup>, Devin Dombrowski<sup>4</sup>, Anthony Riberi<sup>5</sup>, Pranav Pandit<sup>3</sup>, Christine K. Johnson<sup>1,6</sup>, Michael Ziccardi<sup>1</sup>

<sup>1</sup>Karen C Drayer Wildlife Health Center, School of Veterinary Medicine, University of California at Davis, <sup>2</sup>Wildlife Investigations Lab, California Department of Fish and Wildlife, <sup>3</sup>UC Davis, <sup>4</sup>Wildlife Neighbors Database Project, <sup>5</sup>Y3TI, <sup>6</sup>One Health Institute, School of Veterinary Medicine, University of California, Davis, California, USA

# Abstract

Around the world, wildlife populations are increasingly threatened by anthropogenic disturbances, including habitat loss, invasive species introductions, pollution, and disease. Impacts of these disturbances on wildlife highlight the need for far-reaching and coordinated monitoring of threats. Currently, there is limited real-time information on morbidity and mortality events in wildlife. Each year, wildlife rehabilitation centers receive thousands of animals spanning hundreds of different species, which collectively represent a highly diversified dataset with potential for early and enhanced detection of wildlife health threats, particularly in urban and suburban ecosystems. We established a proof of concept study to explore how wildlife rehabilitation centers could enhance the detection of wildlife morbidity and mortality events and contribute to an early warning system for health threats. We developed a web-based application, "Wildlife Morbidity and Mortality Event Alert System" (WMMEAS), which runs in parallel with the free online Wildlife Rehabilitation MD (WRMD) database. The application integrates data in near-real time from a network of centers across California and generates alerts for anomalous wildlife health events. Programmed thresholds are used to detect these events based on a higher than expected number of admissions, which are displayed on an interactive user-interface. In-depth investigations of unusual events are conducted by California Department of Fish and Wildlife in collaboration with the centers. To date, the WMMEAS has identified clusters of morbidity and mortality related to domoic acid toxicity, West Nile virus, Mycoplasma spp., and emerging pathogens, such as Pigeon Paramyxovirus-1 and Sarcocystis calchasi. These results highlight the system's utility for detecting both known and novel threats. Investigations have informed prioritization of monitoring efforts, targeted public education and outreach, and management of clinical cases. We are expanding this proof of concept to include pre-diagnostic clinical classifications and further evaluation of the system's utility for early and enhanced detection of threats.

# VIRALLY-VECTORED VACCINE CANDIDATES AGAINST WHITE-NOSE SYNDROME INDUCE ANTI-FUNGAL IMMUNE RESPONSE IN LITTLE BROWN BATS (MYOTIS LUCIFUGUS)

Tonie Rocke<sup>1</sup>, Jorge Osorio<sup>2</sup>, Bruce Klein<sup>2</sup>

<sup>1</sup>USGS National Wildlife Health Center,<sup>2</sup>University of Wisconsin-Madison

#### Abstract

White-nose syndrome (WNS) caused by the fungus, *Pseudogymnoascus destructans* (Pd) has killed millions of North American insect-eating bats. Currently, methods to prevent the disease are limited. We conducted two trials to assess potential WNS vaccine candidates in wild-caught *Myotis lucifugus*. In a pilot study, we immunized bats with one of four vaccine treatments or PBS as a control and challenged them with *Pd* upon transfer into hibernation chambers. Bats in one vaccine-treated group, that received raccoon poxviruses (RCN) expressing Pd calnexin (CAL) and serine protease (SP), developed WNS at a lower rate (1/10) than other treatments combined (14/23), although samples sizes were small. The results of a second similar trial provided additional support for this observation. Bats vaccinated orally or by injection with RCN-CAL and RCN-SP survived Pd challenge at a significantly higher rate (P = 0.01) than controls. Using RT-PCR and flow cytometry, combined with fluorescent in situ hybridization, we determined that expression of IFN- $\gamma$  transcripts and the number of CD4+ T-helper cells transcribing this gene were elevated (P < 0.10) in stimulated lymphocytes from surviving vaccinees (n=15) compared to controls (n=3). We conclude that vaccination with virally-vectored Pd antigens induced antifungal immunity that could potentially protect bats against WNS.

# PRELIMINARY EVALUATION OF A BACULOVIRUS-EXPRESSED VP2 SUBUNIT VACCINE FOR THE PROTECTION OF WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) FROM EPIZOOTIC HEMORRHAGIC DISEASE

Leela Noronha<sup>1</sup>, Sun Young Sunwoo<sup>2</sup>, Igor Morozov<sup>2</sup>, Jessie Trujillo<sup>2</sup>, Bonto Faburay<sup>2</sup>, Erin Schirtzinger<sup>1</sup>, In Joong Kim<sup>2</sup>, Barbara Drolet<sup>1</sup>, D. Scott McVey<sup>3</sup>, Kinga Urbaniak<sup>2</sup>, David Meekins<sup>2</sup>, Mitchell Palmer<sup>4</sup>, Velmurugan Balaraman<sup>2</sup>, William Wilson<sup>1</sup>, Juergen Richt<sup>2</sup>

<sup>1</sup>United States Department of Agriculture, Agricultural Research Service, Center for Grain and Animal Health Research, Arthropod-Borne Animal Diseases Research Unit, Manhattan, Kansas, <sup>2</sup>Center of Excellence for Emerging and Zoonotic Animal Diseases, and the Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, <sup>3</sup>United States Department of Agriculture, Agricultural Research Service, Center for Grain and Animal Health Research, Manhattan, Kansas, <sup>4</sup>United States Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Infectious Bacterial Diseases Research Unit, Ames, Iowa

# Abstract

Epizootic hemorrhagic disease virus (EHDV) is an arthropod-transmitted RNA virus and the causative agent of epizootic hemorrhagic disease (EHD) in wild and domestic ruminants. In North America, EHD is one of the most important diseases of white-tailed deer (WTD). No commercial licensed vaccine is available in North America, and countermeasures largely rely on vector control. The primary objective of this study was to develop and evaluate a subunit vaccine candidate to reduce clinical disease and viremia in EHDV-infected WTD. Recombinant VP2 (rVP2) outer capsid proteins of EHDV serotypes 2 (EHDV-2) and 6 (EHDV-6) were expressed in baculovirus. Immunogenicity studies in mice and cattle given two doses of EHDV-2 or EHDV-6 rVP2 vaccines produced homologous neutralizing antibodies. In an initial immunogenicity and efficacy study, captive-bred WTD received two doses of EHDV-2 rVP2 or sham vaccine, then were challenged with wild-type EHDV-2 at 30 days post-vaccination. Sham-vaccinees developed clinical signs consistent with EHD, including hyperemia of the oral and ocular mucosa, facial edema, and lymphopenia. Viral RNA was detected in the blood of all sham-vaccinated WTD, with peak RNA viremia occurring at approximately 6 days post challenge. At necropsy, sham vaccinated deer had varying severity of gross and histological lesions consistent with EHD, and viral RNA was detected in a minimum of two of four tissues sampled (lung, liver, spleen and kidney). In contrast, clinical symptoms were not observed in rVP2-vaccinated deer, which also failed to develop PCR-detectable viral RNA in the blood or tissues, and lacked EHD pathology. Here we demonstrate the protective capacity of an EHDV rVP2 subunit vaccine candidate in WTD. This vaccine may be an effective tool in preventing clinical EHD and reducing virus transmission. Moreover, an efficacious single protein-based vaccine would offer DIVA compatibility and a wider safety profile than attenuated live vaccines.

# AL FRANZMANN SPEAKER: CHALLENGES FACING WILDLIFE IN AFRICA AND HOW VETERINARIANS CAN HELP

Pete Morkel

IWVS

# Abstract

Al Franzmann Speaker: Challenges facing wildlife in Africa and how veterinarians can help

## THAT WAS THEN; THIS IS NOW

David Jessup

Wildlife Disease Association

# Abstract

Forty years ago, there were only 5-6 veterinarians working on free-ranging wildlife in North America. The AAWV was founded in July 1979, and in 1981 helped form a Veterinary Section within the WDA. Today there are well over 100 wildlife veterinarians working for State, Provincial and Federal agencies that have the responsibility to manage free-ranging wildlife in North America, and more professionals working in universities, NGO's, or as private practitioners.

Although the dart gun had been invented in the 1960's, only the relatively crude Cap-Chur models were widely available. Only a few drugs for wildlife capture by dart delivery were available. Physical methods of capture, many borrowed from Africa, were in their infancy. Capture myopathy was known but poorly understood, and preventive treatments were not in use. Patient monitoring was limited, and taking basic vital signs were considered 'extras'. A common accepted standard for wildlife captures was a 10% mortality rate.

Wildlife management generally adhered to the dictum "Disease is just a form of compensatory loss and of no significance to most wildlife populations" (Dassman). SCWDS was active, having been founded in the late 1960's. Four State agencies had wildlife pathology labs. The USFWS had the Pautuxent Wildlife Research Center, and Milt Friend was looking for a place for the National Wildlife Health Center. Even when wildlife die-offs were investigated quickly and good samples taken, getting them adequately analyzed was not easy.

Most disease research was done in universities by associated "-ologists". It was these fields, not veterinary medicine, that provided leadership in wildlife health/disease (and WDA). Only a few veterinary schools even had elective courses on wildlife diseases. One Health existed largely in the mind and teachings of Calvin Schwabe. Those interested in wildlife health/disease were generally channeled into one of the very few appropriate PhD or MS programs.

# GENERAL POSTERS

# USE OF FLURALANER AS AN ACARICIDE TO TREAT SARCOPTIC MANGE INFESTATION IN A RED FOX (VULPES)

Robert Adamski<sup>1</sup>, Lynn Miller<sup>2</sup>

<sup>1</sup>New England Wildlife Center, <sup>2</sup>HSUS - South Florida Wildlife Center

#### Abstract

This case study is designed to provide a baseline set of data to determine the safety and efficacy of fluralaner as an acaricide for use in red fox (Vulpes vulpes) when dealing with sarcoptic mange due to Sarcoptes scabei. We will attempt to determine whether a single dose of this medication is effective in treating this parasite. The ultimate goal is to provide an effective, user-friendly and cost-effective treatment option for treating this condition in a captive wildlife rehabilitation setting.

## WILD IN THE CITY: INJURY AND DISEASE IN WILD URBAN PSITTACINES

Patricia Latas<sup>1</sup>, Brooke Durham<sup>2</sup>

<sup>1</sup>Awe Pono Avian Health, <sup>2</sup>SoCal Parrot/REP for Wildlife

# Abstract

Urban psittacine populations may prove important to conservation efforts, and are a deserving taxa of wildlife for legitimate study. Cities may act as refugia for threatened and endangered species, which in turn serve as assurance populations in the event of disaster or local extirpation in the original endemic locale. Urban parrots could provide a source of individuals for re-introduction and translocation programs into native ranges. Southern California supports 13 species of naturalized psittacines; the Red-Crowned Parrot (Amazona viridigenalis), endangered in its endemic range in Mexico, is the most common in San Diego County, California USA. SoCal Parrot is a wildlife rescue, rehabilitation and release facility which works solely with the wild urban psittacines of southern California, and is actively involved in disease surveillance and data collection. We present here common maladies, trauma, and disease entities admitted to the facility for rehabilitation and release, with the ultimate goal to assess the population as a viable and safe source for potential repatriation to endemic ranges. Psittacines admitted into the program were individually assessed using common wildlife veterinary parameters and disease testing. Over 5 years of data collection, anthropogenic trauma was the most common finding. Potential infectious disease or unknown illness accounted for only a small percentage of admissions. There were no common infectious agents of concern detected. The emerging field of urban wildlife biology needs to include wildlife disease as a major factor in population and biodiversity studies and urban parrots are easily accessible and free from regulatory constraints. Urban psittacine species from Southern California are unlikely to labor contagious disease and are good candidates for repatritiation; although further and more detailed surveillance is recommended.

# WILD PSITTACINE REHABILITATION

Patricia Latas

Awe Pono Avian Health

# Abstract

In many parts of the world, wildlife rehabilitators work with psittacine species, yet little is known or published regarding disease, injury, or standards or goals of care for these species. Involvement with veterinarians and wildlife disease experts is often unavailable. A recent, informal survey of people involved with rescue, rehabilitation and release of wild psittacines highlights the deficits of knowledge and interaction. This is the first published survey of wildlife carers and rehabilitators specifically involving wild psittacines. It is the goal of this presentation to make the needs and concerns known and to offer bridging of disciplines to assist evidence-based data collection and eventual successful release to the wild. Results of this survey will be presented here with suggestions to improve the over-all success of release and recruitment of individual birds into a functioning population. A multi-lingual Survey Monkey questionnaire was sent to wildlife carers and rehabilitators are species. Non-statistical and informal results were compiled for informational resources. Common problems facing wild psittacine rehabilitators across the globe included lack of veterinary resources, similar injury and and etiological entities. With accelerating diminishment of psittacine populations due to anthropogenic impact, it is imperative that all members of psittacine conservation community work in tandem to ameliorate ignorance and failure.

# BAYESIAN PHYLOGENETIC ANALYSIS OF AVIPOXVIRUSES FROM NORTH AMERICAN WILD BIRDS DEMONSTRATES NEW INSIGHTS INTO HOST-SPECIFICITY AND INTERSPECIES TRANSMISSION

Amanda MacDonald<sup>1</sup>, Daniel Gibson<sup>1</sup>, John Barta<sup>1</sup>, Rebecca Poulson<sup>2</sup>, Justin Brown<sup>3</sup>, Andrew Allison<sup>4</sup>, Nicole Nemeth<sup>2</sup>

<sup>1</sup>University of Guelph, <sup>2</sup>University of Georgia, <sup>3</sup>Penn State, <sup>4</sup>University of Florida

# Abstract

Avian poxvirus is a highly contagious viral pathogen of wild and domestic birds. These viruses are found globally; however, host-specificity related to genetic differences in avian poxviruses and a broader understanding of phylogenetic relationships need further exploration. The objective of this study was to perform Bayesian phylogenetic analysis to compare avian poxvirus isolates among different avian taxa within Ontario and the United States to isolates previously derived from birds in six continents to determine strain relatedness. PCR analysis targeting the highly conserved 4b core protein was used to amplify poxvirus DNA from 82 birds of varying species. Following sequencing, a phylogenetic analysis was performed, including these new 82 isolates and 70 publicly available sequences. A total of 12 avian species were represented; the most common were wild turkeys (Meleagris gallopavo), mourning doves (Zenaida macroura), and American crows (Corvus brachyrhynchos). Bayesian phylogenetic analyses of these, and publicly available sequences, revealed statistically significant monophyletic clades based on genetic distances of sequences within and between observed clades. Genetic variation within the fowlpox clade was low compared to the canarypox clade. Host and geographic origins of viral isolates revealed overall clustering of viral strains within avian species, with a few exceptions. No genetic differences were observed between viruses from Canada and the United States within individual species. Most new isolates clustered with their respective reference strain, suggesting poxvirus infections are often restricted to a particular taxa. Sporadic incidences of inter-specific poxvirus infection, however, demonstrate evolving strains with the potential to infect >1 host, important for wildlife management strategies targeting the prevention of outbreaks in species of vulnerable conservation status. This study provides new phylogenetic data on poxviruses from birds in Canada and the United States, contributes to the limited understanding of host range, and can be used in future phylogenetic studies.

# THE ROLE OF THE WORLD ORGANISATION FOR ANIMAL HEALTH (OIE) IN WILDLIFE DISEASE SURVEILLANCE

Jonathan Sleeman

USGS National Wildlife Health Center and OIE National Focal Point for Wildlife for the United States

# Abstract

The need to fight animal diseases at the global level led to the creation of the Office International des Epizooties (World Organisation for Animal Health; OIE) through an international agreement signed in 1924. The OIE is the intergovernmental organization responsible for improving animal health and welfare worldwide with 182 Member Countries and regional offices on every continent. The OIE recognizes the threats to public, animal, and environmental health from wildlife diseases, and encourages all countries to increase capacity to conduct surveillance, early detection, and initiate appropriate responses to outbreaks and spread of diseases in wildlife. Activities of the OIE related to wildlife diseases include a standing Working Group on Wildlife of scientific experts, development of science-based standards related to disease risks at the wildlife, domestic animal, and human interface, support to Member Countries to protect animal health including wildlife and biodiversity, and surveillance and notification of wildlife diseases through the global OIE information system WAHIS-Wild. Each Member Country is encouraged to appoint a National Focal Point for Wildlife (NFPW) with several responsibilities, including:

1. Establish a network of wildlife experts within his/her country or to communicate with the existing network, and to facilitate communication among several authorities where responsibility is shared;

2. Under the authority of the OIE Delegate of his/her country, support the optimal collection and submission of wildlife disease information to the OIE through WAHIS-Wild. Specifically, there are currently 53 non-OIE listed wildlife diseases of interest (http://www.oie.int/wahis\_2/public/wahidwild.php/Diseaseinformation/popup/diseaselist), and the NFPW is responsible for providing information to the Member Country's Delegate for submission of the annual voluntary report for wildlife to the OIE concerning detection of these diseases. Reporting of wildlife diseases is important to build situational awareness regarding wildlife health, build national knowledge capacity, increase coordination among agencies, and integrate wildlife health data into other surveillance frameworks.

# SEROPREVALENCE OF WEST NILE VIRUS IN RUFFED GROUSE (BONASA UMBELLUS) POPULATIONS IN EASTERN CANADA

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# Abstract

West Nile virus is a widespread virus, transmitted through the bite of infected mosquitoes. Between 2017-2018, 180 dead wild birds were tested for West Nile virus by the Canadian Wildlife Health Cooperative and 48.9% were positive, including the first West Nile virus-positive wild turkey in Ontario. Susceptibility to disease and population impacts associated with West Nile virus vary between galliform species, and in the United States, population-level impacts have been reported for highly susceptible species (e.g. sage grouse and ruffed grouse). In Canada, ruffed grouse (Bonasa umbellus) are an economically valuable gamebird; however, studies concerning the potential for population impacts due to West Nile virus are lacking. This study aimed to determine the prevalence and distribution of West Nile virus antibodies in populations of ruffed grouse within Ontario and Québec, Canada. During 2018-2019 hunting season, blood-soaked nobuto filter paper strips were collected from 86 ruffed grouse in Québec and 130 in Ontario. Nobuto strip eluate was tested for West Nile virus-neutralizing antibodies by the plaque reduction neutralization test. Samples with >80% neutralization were further tested to distinguish between West Nile virus and St. Louis encephalitis virus by a  $\geq$ 4-fold PRNT80 titer. Seroprevalence and distribution from across two Canadian provinces will be reported at time of conference. The data provided by this study will aid in future studies, and also conservation and management strategies of ruffed grouse populations. Also, given the risk of West Nile virus to other wildlife and humans, these data can assist in monitoring virus activity, especially in the face of global climate change.

# LOOK AND LEARN: INVESTIGATIONS INTO OCULAR ANATOMY AND PATHOLOGY IN LUMHOLTZ'S TREE-KANGAROO (DENDROLAGUS LUMHOLTZI)

Amy Shima<sup>1</sup>, Linda Johnson<sup>2</sup>, Lee F. Skerratt<sup>1</sup>, Caroline Zeiss<sup>3</sup>

<sup>1</sup>University of Melbourne, Faculty of Veterinary and Agricultural Science, <sup>2</sup>James Cook University, College of Public Health, Medicine and Veterinary Science, <sup>3</sup>Yale University School of Medicine-Department of Comparative Medicine

# Abstract

Lumholtz's tree-kangaroo (Dendrolagus lumholtzi) is one of two species of tree-kangaroos found in far north Queensland, Australia. There have been recent reports of blindness in free-ranging animals and a viral or neurotoxic cause has been suggested despite a lack of diagnostic investigations. Indeed there is little information about ocular anatomy and pathology in any species of tree-kangaroo. Hence we investigated normal ocular anatomy and pathologi in 88 Lumholtz's tree-kangaroos, utilizing 1) ophthalmic examination of 39 live animals and 2) histopathologic examination of 94 eyes from 49 road-killed carcasses. Tree-kangaroos were found to have a typical vertebrate eye with putative dichromatic color vision (yellow-red). Our study found no evidence of widespread blindness in free-ranging tree-kangaroos with pathology, seen in only 5.4% of animals, consisting of mild, nonspecific ocular lesions due to traumatic injury, protozoal infection and bacterial infection. Toxoplasmosis was implicated in ocular lesions in three animals. We saw no evidence of toxic optic neuropathy. Further work is needed to develop vision testing protocols appropriate for the species, as well as targeted investigation of animals reported to be suffering from central blindness of unknown origin.
# PREVALENCE, GENETIC DIVERSITY AND PARATENIC HOSTS OF DRACUNCULUS INSIGNIS IN MESO-MAMMALS FROM DI-LANE PLANTATION, GEORGIA

Christopher Cleveland<sup>1</sup>, Mark Eberhard<sup>2</sup>, Kayla Garrett<sup>1</sup>, Alec Thompson<sup>1</sup>, Liandrie Swanepoel<sup>1</sup>, Elizabeth Miller<sup>3</sup>, Odin Stephens<sup>3</sup>, Michael Yabsley<sup>1</sup>

<sup>1</sup>University of Georgia/Dept. of Population Health/Southeastern Cooperative Wildlife Disease Study, <sup>2</sup>Centers for Disease Control and Prevention/Retired, <sup>3</sup>USDA APHIS Wildlife Services

## Abstract

The prevalence and diversity of parasitic nematodes in wildlife has been well studied for certain species, yet for others there exist considerable gaps in knowledge. The parasitic nematode, Dracunculus insignis, infects North American wildlife and past research has led to increased understanding of the potential host diversity and transmission of the closely related human Guinea worm, D. medinensis (currently the focus of a global eradication program). Many definitive hosts have been documented for D. insignis, however, the life-cycle has only been studied in laboratories and not proven in the field. Additionally, only one phylogenetic study has been conducted on D. insignis from Canada. The goal of this study was to investigate the prevalence of Dracunculus infections among wildlife at a single site (Di-Lane Plantation) in the southeastern United States, evaluate the genetic diversity of parasites at this site, and investigate the role of paratenic hosts in transmission. Over the course of 3 years, we sampled 228 meso-mammals, reporting an overall prevalence of infection with Dracunculus insignis of 20% (46/195). Sampling of 63 individual fish of a single species and 68 frogs representing 5 species from water bodies in the same geographic area as infected meso-mammals resulted in recovery of D. insignis third stage larvae (L3s) from 2 species of amphibians, but all fish sampled were negative. The phylogenetic analysis of the partial cytochrome-c oxidase I (COI) gene shows very little diversity of Dracunculus at Di-Lane; however, we did recover a single nematode from a Virginia opossum (Didelphis virginiana) that falls outside of the D. insignis clade, more closely aligns with D. lutrae, and may represent an undescribed species. This work increases life-cycle knowledge on a previously undocumented transmission pathway in nature at a single site with endemic transmission of D. insignis among raccoons and opossums.

# RICKETTSIA SPECIES IN TICKS COLLECTED FROM WILD PIGS (SUS SCROFA) AND PHILIPPINE DEER (RUSA MARIANNA) ON GUAM, MARIANNA ISLANDS, USA

Christopher Cleveland<sup>1</sup>, Liandrie Swanepoel<sup>2</sup>, Erin Box<sup>2</sup>, Anthony DeNicola<sup>3</sup>, Michael Yabsley<sup>2</sup>

<sup>1</sup>University of Georgia, <sup>2</sup>University of Georgia/Dept. of Population Health/Southeastern Cooperative Wildlife Disease Study, <sup>3</sup>White Buffalo, Inc.

## Abstract

The prevalence and diversity of ticks on wildlife species on Guam is understudied, as to date no work has been conducted on the infection of these ticks with Rickettsia (obligate intracellular pathogens that use a variety of ectoparasites as vectors and can cause disease in humans, domestic animals, and wildlife species). The goal of our study was to investigate the presence of Rickettsia species on the island of Guam by testing ticks removed from Philippine deer (Rusa marianna) and wild pigs (Sus scrofa). Increasing numbers of these species have led to increased interactions with humans, including hunting, highlighting the importance of studies on vector prevalence and associated zoonotic pathogens. In this study, ticks were removed from Philippine deer and wild pigs in March and April of 2015 and tested for Rickettsia spp. using nested PCR. Overall, a low prevalence of Rickettsia spp. were detected (5.4% (6/112 ticks)). Ticks removed from wild pigs were identified as Amblyomma breviscutatum, one of which was positive for Rickettsia ambylommatis-like species. Ticks recovered from Philippine deer were identified as Rhipicephalus microplus, and three were positive for a Rickettsia species; two with R. amblyommatis and one with R. felis. Rickettsia felis is a zoonotic pathogen in the spotted fever group of Rickettsia. This study confirms the occurrence of Rickettsia in ticks on Guam and highlights the presence of human pathogenic species such as R. felis.

# LYMPHOPROLIFERATIVE DISEASE VIRUS IN WILD TURKEYS (MELEAGRIS GALLOPAVO) FROM MANITOBA AND QUEBEC, CANADA

Amanda MacDonald¹, Michelle McKay², John Barta¹, Stephane Lair³, Nicole Nemeth⁴, Neil Pople⁵, Frank Baldwin⁵

<sup>1</sup>University of Guelph, <sup>2</sup>Trent University, <sup>3</sup>Universite de Montreal, <sup>4</sup>University of Georgia, <sup>5</sup>Government of Manitoba

## Abstract

In Canada, the wild turkey (Meleagris gallopavo) historically occupied a range that included the southern-most parts of Ontario and Quebec; however, was extirpated in the early 1900s due to unregulated harvest. Re-introductions and range expansion subsequently re-established populations that currently support hunting seasons, making wild turkeys a valued and economically important game species in both provinces. Lymphoproliferative disease virus (LPDV), a relatively newly described avian retrovirus in wild turkeys, has been reported in the United States (U.S.) and Ontario, Canada; however, its status in Manitoba and Quebec is unknown. The objectives of this study were to assess wild turkey samples from Manitoba and Quebec for LPDV and reticuloendotheliosis virus (REV), and to perform a phylogenetic analysis to determine strain relatedness to wild turkey LPDV sequences from Ontario and the U.S. Tissues from 69 wild turkeys (Manitoba, n=65 from trap and transfer birds; Quebec, n=4 from diagnostic cases) were tested for LPDV and REV by PCR. Sequencing was performed on LPDV-positive samples and along with publicly available sequences from Ontario and the U.S., were used to perform Bayesian phylogenetic analysis. LPDV was detected in 34.8% from both provinces combined (24/69; Manitoba, 20/65; Quebec, 4/4) of samples. One of the turkeys from Quebec had gross and microscopic lesions consistent with LPD, including masses on the head and neck characterized by multinodular accumulations of highly pleomorphic lymphocytes in the dermis. A concurrent infection of LPDV and REV was detected in another Quebec wild turkey. LPDV sequences from Manitoba and Quebec were genetically similar to other North American strains and did not group into separate clades. This study provides the first evidence of LPDV circulation in Manitoba and Quebec, including the first known clinical case in Canada. The data provided will contribute to future studies on LPDV surveillance and phylogenetics in wild turkeys.

# SURVEILLANCE FOR BAYLISASCARIS SP. IN BLACK BEARS (URSUS AMERICANUS), STRIPED SKUNKS (MEPHITIS MEPHITIS), AND GROUNDHOGS (MARMOTA MONAX) IN PENNSYLVANIA, USA

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<sup>1</sup>Pennsylvania State University-Altoona, <sup>2</sup>Department of Veterinary and Biomedical Sciences, Pennsylvania State University, <sup>3</sup>St. Francis University, <sup>4</sup>Pennsylvania Game Commission, <sup>5</sup>United States Department of Agriculture, Animal Plant Health Inspection Service, Wildlife Services, <sup>6</sup>University of Georgia/Dept. of Population Health/ Southeastern Cooperative Wildlife Disease Study, <sup>7</sup>South

#### Abstract

Baylisascaris procyonis, the raccoon roundworm, is a well-studied nematode that causes severe disease in paratenic hosts associated with larval migrans. Visceral, ocular, or neural larval migrans associated with B. procyonis have been reported in a wide diversity of animals and humans. Although other New World species of Baylisascaris have been identified, the biology and distribution of these nematodes is much less defined. In Pennsylvania, B. laevis, B. columnaris, and B. transfuga have been reported in groundhogs, striped skunks, and black bears, respectively; however, the prevalence of infections in these definitive hosts is unknown. The objective of this study was to survey black bears, striped skunks, and groundhogs in Pennsylvania for Baylisascaris sp. During 2017-2018, gastrointestinal tracts were collected from 41 black bears, 93 striped skunks, and 242 groundhogs from Pennsylvania. Intestinal contents were examined for Baylisascaris sp. using a 1-mm sieve. Feces from each animal was also examined for Baylisascaris sp. eggs by centrifugal floats. A high prevalence of Baylisascaris sp. was found in black bears (21/41; 51%) with an average burden of 10 worms (range: 1-55). Of these infections, 57% were patent with an average of 57 eggs/gram of feces (range: 1-371). Similarly, striped skunks had a high prevalence of Baylisascaris sp. (41/93; 44%) with an average burden 8 worms (range: 1-50). Eighty percent of the skunk infections were patent with an average of 140 eggs/gram of feces (range: 3-610). No Baylisascaris sp. were identified in the groundhog intestines. These results indicate black bears and striped skunks in Pennsylvania are commonly infected with Baylisascaris sp. While infection in groundhogs has been reported, these data indicate the prevalence is low. Although less is known about B. columnaris and B. transfuga, both species can cause larval migrans. Consequently, larvae detected in paratenic hosts should not be assumed to be B. procyonis.

# CHALLENGES FOR KEEPING THE CRITICALLY ENDANGERED ARCTIC FOX (VULPES LAGOPUS) IN FENNOSCANDIA HEALTHY

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### Abstract

On mainland Fennoscandia, the arctic fox (Vulpes lagopus) is considered critically endangered. Joint conservation efforts in Norway, Sweden and Finland have increased the population from less than 50 adult individuals in 2000 to approximately 300 individuals in 2018. Between Norway and Sweden alone, over 2.1 million USD are used for conservation of this species per year. Actions include supplemental feeding, captive breeding/release and red fox (Vulpes vulpes) culling, as well as population monitoring.

The arctic fox management plans have outlined the need for health monitoring since 2003. Despite this, management authorities have lacked the resources to start a comprehensive health program across the Fenno-Scandinavian countries. Because the majority of arctic fox individuals live in small, partially isolated subpopulations in mountain fragments surrounded by forest, they are vulnerable to local extinctions from disease outbreaks. Close interaction between other canid species [e.g. red fox, domestic dogs] strongly increases risk for disease-mediated population decline. Canine parvovirus, canine adenovirus 1, canine distemper, and sarcoptic mange are all diseases which could result in rapid extinction of local arctic fox populations. In addition, new diseases and pathogens are continually being discovered in Fennoscandia (e.g. French heartworm, Angiostrongylus vasorum). Because mange outbreaks have occurred in the Swedish arctic fox populations, emergency plans and transboundary communication exist for this disease; however, plans for other disease threats have yet to be developed.

As a first step to gaining funding and political support, we have begun a description of potential disease threats and corresponding management efforts based on literature and current disease knowledge/risk of disease transmission in Fennoscandia. With this presentation, we outline the management policy and conservation efforts in Fennoscandia thus far, discuss the potential impacts of outbreaks of different infectious diseases, and present our recommendations for health management efforts in the future.

# NERVE GROWTH FACTOR RECEPTOR - TROPOMYOSIN RECEPTOR KINASE A: A POTENTIAL THERAPEUTIC TARGET PATHWAY IN TASMANIAN DEVIL FACIAL TUMOUR DISEASE?

Tammy Shadbolt<sup>1</sup>, Ken Smith<sup>2</sup>, Imelda McGonell<sup>2</sup>

<sup>1</sup>Zoological Society of London, <sup>2</sup>Royal Veterinary College

# Abstract

The Tasmanian devil (Sarcophilus harrisii) is currently threatened with disease induced extinction due to a recently emerged transmissible cancer, Tasmanian devil facial tumour (DFT). On clinical and histopathological examination DFT displays hallmarks of an aggressive malignant neoplasm with a high metastatic rate to visceral organs. The mechanisms of metastasis are largely unknown but can be better understood by investigating the expression of genes that may endower tumour cells with the potential to migrate. It was hypothesised that DFT would express genes considered important drivers of metastasis in other cancers of neural crest lineage. Further, that chemical inhibition studies in vitro would reveal a potential functional role for these expressed genes in DFT cell migration. Tumour tissue samples were collected during fieldwork with the University of Tasmania and cell lines obtained through the Tasmanian government. Gene expression was revealed using quantitative polymerase chain reaction techniques. Cell migration was investigated in vitro using scratch assays and molecular pathways manipulated using chemical inhibition. Novel candidate genes nerve growth factor receptor (NGFR) and tropomyosin receptor kinase A (TrkA) were highly expressed in DFT tissues and cells. Chemical inhibition of the NGFR-TrkA receptor complex resulted in notably altered DFT cell morphology and significantly reduced cell migration in vitro. Results suggest that the expression of NGFR-TrkA by DFT cells and signaling through the receptor complex plays a functional role in promoting tumour metastasis. It is proposed that further research into the NGFR-TrkA pathway could be vital for understanding the aggressive metastatic nature of DFT and may reveal promising therapeutic targets in cancer research.

# TICKS AND TREE LINE: MONITORING AND PREPARING FOR CLIMATE CHANGE IMPACTS ON ZOONOSES IN SUBARCTIC CANADA

Heather Fenton<sup>1</sup>, Brett Elkin<sup>1</sup>, Stephanie Behrens<sup>1</sup>, Suzanne Carriere<sup>1</sup>, Dean Cluff<sup>1</sup>, Heather Hannah<sup>1</sup>, Emily Jenkins<sup>2</sup>, Allicia Kelly<sup>1</sup>, Susan Kutz<sup>3</sup>, Nic Larter<sup>1</sup>, Robbin Lindsay<sup>4</sup>, Brent Wagner<sup>2</sup>, Carl Ribble<sup>3</sup>

<sup>1</sup>Government of the NWT, <sup>2</sup>Univeristy of Saskatchewan, <sup>3</sup>University of Calgary, <sup>4</sup>Public Health Agency of Canada

# Abstract

As the North is experiencing rapid environmental and land-use changes, there are anticipated changes in the distribution and populations of biting arthropods. The Northwest Territories (NWT) amasses roughly 1.4 million km2 (442 000 square miles) of land with a human population density of 0.1 people per square mile scattered among 33 communities with 11 official languages. Performing statistically-relevant surveillance programs for any diseases can be challenging and costly in such a vast landscape that is often only accessible by boat or air. There is limited baseline information on current distributions of arthropod species in the NWT and pathogens that they could be carrying. There is interest among health professionals and the public about arthropods (e.g. ticks and mosquitoes) and pathogens they could carry that could harm themselves (e.g. West Nile virus and related California serogroup viruses), pets (e.g. tick-borne rickettsia and West Nile virus), and the wildlife (e.g. potential impacts of winter tick or Dermacentor albipictus on moose and insect harassment of caribou) in terms of food security, food safety, and conservation. This project aims to summarize current baseline information within the NWT, formally evaluate the risk of introduction of target pathogens (Borrelia burgdorferi and West Nile virus) into the NWT, geographical expansion of known pathogens and arthropods in response to climate change (e.g. Francisella tularensis the causative agent of tularemia and winter tick), and identify appropriate priorities for better use of shared resources for surveillance activities, responding to community-based concerns, strengthen relationships and communication among departments, collaborative agencies, university and laboratory partners, and plan for the detection of these pathogens in terms of risk communication strategies, diagnostic, and personnel capacity.

# EPIDEMIOLOGY OF INFECTIOUS PNEUMONIA-ASSOCIATED PATHOGENS IN ENDANGERED PENINSULAR BIGHORN SHEEP

Jessica Sanchez<sup>1</sup>, Ben Gonzales<sup>2</sup>, Steve Torres<sup>2</sup>, Brandon Munk<sup>2</sup>, Lora Konde<sup>2</sup>, Janene Colby<sup>3</sup>, Annette Roug<sup>4</sup>, Christine K. Johnson<sup>1,5</sup>

<sup>1</sup>Karen C. Drayer Wildlife Health Center, School of Veterinary Medicine, University of California at Davis, <sup>2</sup>Wildlife Investigations Lab, California Department of Fish and Wildlife, <sup>3</sup>California Department of Fish and Wildlife, South Coast Region, <sup>4</sup>Utah Division of Wildlife Resources, <sup>5</sup>One Health Institute, School of Veterinary Medicine, University of California, Davis, California, USA

#### Abstract

Polymicrobial, epizootic pneumonia is a source of mortality and decreased lamb recruitment in Peninsular bighorn sheep (Ovis canadensis nelsoni) throughout their range. Epizootic pneumonia originally entered the Peninsular bighorn sheep population through contact with domestic sheep (Ovis aries) but can be maintained by carrier bighorn sheep (BHS) for years, causing intermittent epizootics. BHS pneumonia is a complicated disease process that has been historically difficult to unravel, involving co-infection with several infectious pathogens, environmental and immune factors, and host behavior. Numerous management efforts in other BHS populations have failed to prevent or control pneumonia outbreaks, highlighting the multifactorial nature of this disease. Recent research on BHS populations across the western United States suggests that Mycoplasma ovipneumoniae is a primary pathogen associated with many pneumonia outbreaks. Mycoplasma ovipneumoniae may act to decrease immune function, exacerbating the effects of other pathogens such as Pasteurella spp. or respiratory viruses. This study is the first to investigate associations between infectious disease exposure and population health of BHS in the Peninsular Ranges. Samples were collected from Peninsular BHS (n = 839) from 1981-2017 and tested for varying subsets of 15 pathogens. Data were also collected on population demographics at regular intervals. Pathogen-specific spatial and temporal clusters that were higher or lower than expected were identified for each of the 9 recovery regions within the study area. Utilizing multiple regression models, we identified significant relationships between pathogen prevalence and the population demographics of sheep across the region. These results will be used to inform BHS management and conservation, as well as identify mechanisms of disease transmission that are relevant to other species. The habitat range of BHS continues to overlap with areas of high utilization by humans and domestic livestock, increasing the risk of pathogen transmission among host species and complicating the control of this devastating disease.

# RENAL AND VAGINAL CALCULI IN A FREE-RANGING LONG-BEAKED COMMON DOLPHIN (DELPHINUS CAPENSIS)

Kerri Danil¹, Sophie Dennison², Elyse Wurster¹³, Michael Garner⁴, Judy St. Leger⁵

<sup>1</sup>NOAA/NMFS/Southwest Fisheries Science Center, <sup>2</sup>TelVet Imaging Solutions, <sup>3</sup>Lynker Technologies, <sup>4</sup>Northwest ZooPath, <sup>5</sup>SeaWorld

# Abstract

Nephrolithiasis with vaginal calculus was identified in a stranded free-ranging long-beaked common dolphin (Delphinus capensis). Necropsy examination of the sexually mature D. capensis revealed multiple bilateral nephroliths and a 6.4 cm x 4.1 cm x 9.2 cm vaginal calculus weighing 182 g. Concurrently, the animal had a lytic and sclerotic lesion of the lumbar vertebrae with fusion of associated spinous processes. The extracted nephroliths ranged in size from 0.1 cm to 0.4 cm and were composed of 100% ammonium urate, which has been found in captive dolphin populations, while the vaginolith consisted of struvite and calcium carbonate. This disparity of composition suggests that the etiology of the two conditions was not directly related. No infectious agents were identified histologically or by calculi culture. Additionally, morbillivirus PCR, Brucella PCR, biotoxin analysis, and heavy metal concentrations were within normal limits. The vertebral lesion is considered incidental. However, the composition of the vaginolith suggests that a bacterial vaginitis could have served as the predisposing condition. Staphylococcus sp. was isolated on uterine culture and Staphylococcus sp. producing urease has been associated with formation of struvite calculi.1 Although previously reported vaginoliths in common dolphins have been linked with fetal remains, this case is more similar to struvite vaginoliths reported in other species.2-6 Renal lesions included chronic tubulointerstitial nephritis with tubular degeneration and loss likely secondary to the presence of the nephroliths. The etiology of ammonium urate nephrolithiasis in managed care is linked to diet and age.7-9 If environmental changes cause a shift in prey species, the risk of nephrolithiasis in free-ranging cetaceans could increase. Careful surveillance for nephroliths in free-ranging populations should be considered by researchers.

# ANTIMICROBIAL RESISTANCE IN COMMON SEALS (PHOCA VITULINA); A MARINE CANARY IN THE COALMINE?

Paul Duff<sup>1</sup>, Manal AbuOun<sup>1</sup>, Steve Bexton<sup>2</sup>, Muna Anjum<sup>1</sup>, Christopher Teale<sup>1</sup>

<sup>1</sup>Animal and Plant Health Agency, <sup>2</sup>Royal Society for the Prevention of Cruelty to Animals

### Abstract

The Animal and Plant Health Agency (APHA) Diseases of Wildlife Scheme (DoWS) delivers, in collaboration with other agencies, national wildlife disease surveillance in England and Wales. DoWS, working with a wildlife hospital which specialises in rescuing sick common seals, and a government team specialising in anti-microbial resistance (AMR) have together been monitoring bacterial pathogens and their antimicrobial resistance patterns, in stranded common seals off the East coast of England for 7 years. Preliminary results were published in 2016 (Duff et al.) and provided evidence that some bacterial pathogens in a small number of seals, are directly or indirectly derived from man. Some of these in turn have acquired resistance to an extensive range of antibiotics including 'highest priority antibiotics'. Contamination of the marine environment through discharge of sewage into the sea presents the most likely route of exposure for seals.

Wildlife disease, conceivably, may provide early information allowing the detection of change in our environment; a modern version of a veritable 'canary in the coalmine'. Is AMR in seals caused by marine pollution from sewage discharges an example? Some other possible 'wild canaries' over the past 60 years from the global wildlife disease archive are listed and compared in order to try to assess if wildlife disease really can provide an effective, sensitive, early warning system capable of detecting change in the environment before their detection by other, more orthodox, means. If instances of wildlife disease have validity as examples of modern canaries in the coalmine – so what, can they usefully inform us?

Ref: Duff et al (2016) Klebsiella pneumoniae of suspected human origin from free-living common seals on the east coast of England, Veterinary Record, 2016, doi: 10.1136/vr.104018

### ENDOGENIZATION OF A PROSIMIAN RETROVIRUS DURING LEMUR EVOLUTION

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# Abstract

Studies of viruses that evolved with lemurs provide an opportunity to understand the basal traits of primate viruses and provide the evolutionary context of host-virus interactions. In contrast to mainland primates in which many types of viruses have been identified, there is very little known about lemur viruses. In order to characterize novel lemur viruses, we performed deep sequencing of sera collected from 8 species of lemurs in the Saint Louis Zoo and identified DNA sequencing reads mapping to the Retroviridae family in Coquerel's sifaka (Propithecus coquerel). Using PCR with up to 3x fold coverage, we verified the complete genome sequence of a retrovirus, named prosimian retrovirus 1 (PSRV1). Phylogenetic analyses indicate that PSRV1 is a gamma-like betaretrovirus basal to the other primate betaretroviruses and most closely related to simian retroviruses. We then used PCR to evaluate DNA from lemur liver, screening for PSRV1 using primers that target the pol region. A hallmark of endogenized retroviruses is that the integrated viral genome is transmitted vertically through the germline and expected to be detected in the DNA of all tissue types. We found PSRV1 DNA in 8 out of 8 livers from P. coquerel, but did not find PSRV1 DNA in livers from black lemurs, ringtailed lemurs, or black-and-white ruffed lemurs. This suggests that PSRV1 is a novel endogenous retrovirus specific to P. coquerel. The characterization of novel viruses could lead to a new model of lemur disease burdens, allowing us to better understand the cause of lemur mortality and develop more effective ways to prevent, diagnose, and treat diseases. These findings also have implications for both human and animal health in that the acquisition of a gamma-type env gene in place of a beta-type env within an endogenized betaretrovirus could facilitate a cross-species jump between vertebrate class hosts.

# WILDLIFE DISEASE SURVEILLANCE IN COOK COUNTY, ILLINOIS: PROMOTING ANIMAL AND PUBLIC HEALTH THROUGH DIAGNOSTIC PATHOLOGY AND COLLABORATIVE RESEARCH

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<sup>1</sup>University of Illinois Zoological Pathology Program, <sup>2</sup>Cook County Forest Preserve District, <sup>3</sup>Cook County Animal Control

## Abstract

Many wildlife species successfully utilize and have colonized urban and suburban spaces, increasing potential for interactions and disease transmission among humans, wildlife and domestic animals. Yet, much of our understanding of wildlife diseases comes from studies within larger contiguous natural areas where disease dynamics likely differ from those in urban settings with fragmentation of habitats and increased contact rates. Long term studies of urban and suburban wildlife are critical for understanding the pathogenesis and epidemiology of infectious diseases, providing recommendations for wildlife and land management, and informing local and state agencies of potential public health risks. For over 20 years, the Environmental Impact Research Group (EIRG), which includes wildlife biologists, veterinarians, and pathologists, has investigated wildlife disease ecology in Cook County, Illinois. This collaborative effort encompasses passive and active surveillance of over 1 million acres of industrialized regions to uninhabited green spaces and numerous species of animals. As part of this program, complete necropsies have been conducted on thousands of wild animals including bird, amphibian, reptile, and fish species in addition to various mesocarnivores, rabbits, and deer. Several focused research projects have developed from this surveillance program. For example, evaluation of canine distemper epizootics over time in multiple sympatric mesocarnivores is enhancing our knowledge of this viral disease in urban wildlife, enabling local animal control officials to better mitigate spillover into domestic animal populations. Surveillance has also aided public health officials including the diagnosis and characterization of a tularemia outbreak in free-ranging rabbits, and more recently fish health monitoring and ecotoxicology studies in inland county lakes used for recreational fishing. Through ongoing surveillance, diagnostics, and prospective research programs, wildlife disease ecology in Cook County will be better understood as will the potential impacts on wild populations, and implications to the health of humans, animals, and the diverse county ecosystems.

# ANALYZING ANTHROPOGENIC CONTAMINANT CONCENTRATIONS AND PATHOLOGIC TRENDS IN STRANDED CETACEANS

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## Abstract

Anthropogenic contaminants are frequently released into coastal marine environments from activities such as manufacturing, pesticides, and burning of fossil fuels in addition to their uses in plastics, detergents, and other common household chemicals. Within the marine environment, such anthropogenic contaminants tend to biodegrade slowly, bioaccumulate in organisms, and can negatively affect marine wildlife health. With this study, we aim to help expand the knowledge base concerning concentrations and potential biological effects of anthropogenic contaminants in stranded cetaceans. Liver samples taken from cetaceans that stranded in two different locations along the southeastern coast of the United States during 2016–2018 were measured for concentrations of polychlorinated biphenyl (PCB) 1268 congeners using gas chromatography-mass spectrometry, and for concentrations of 12 inorganic elements (Hg, Pb, Cd, As, Zn, Se, Mn, Mb, Fe, Cu, Co, Tl) using inductively coupled plasma-mass spectrometry. Overall, we analyzed samples from 35 cetaceans that stranded along the central east coast of Florida (n=14) and the coast of North Carolina (n=21). Species evaluated include Tursiops truncatus, Kogia breviceps, Mesoplodon europaeus, Physeter macrocephalus, Ziphius cavirostris, Globicephala macrorhynchus, Balaenoptera acutorostrata, and Megaptera novaeangliae. For each individual animal, complete gross and microscopic pathologic exams were conducted. To evaluate for potential adverse biological effects related to exposure to these contaminants, the pathology data were evaluated for correlations with concentrations of the various contaminants using the Kruskal-Wallis one-way analysis of variance. Additionally, contaminant concentrations were compared between various demographic variables (i.e., age class; diet; location stranded) using the Mann-Whitney-Wilcoxon test with a Bonferroni correction. Data are being analyzed and results will be presented. Data from this study provide information on concentrations and potential sublethal effects of exposure to certain contaminants that could have chronic effects at the population level and at the individual level for cetaceans inhabiting coastal marine ecosystems.

# THE PHARMACOKINETICS AND ESTIMATED DRUG WITHDRAWAL TIME OF FLURALANER IN AMERICAN BLACK BEARS (URSUS AMERICANUS)

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<sup>1</sup>Wildlife Center of Virginia, <sup>2</sup>North Carolina State University

## Abstract

The increasing incidence of sarcoptic mange in American black bears necessitates practical treatment options for free-ranging animals. Fluralaner is an ectoparasitic prevention drug used in domestic animals and has proven effectiveness against Sarcoptes spp. mites. Because of a half-life of fluralaner in dogs (12-15 days), a single oral dose may be sufficient to eliminate all life stages of the mites. This is an important advantage over other medications that must be administered more frequently and/or at high doses. In this study, we administered one oral dose (25mg/kg) of fluralaner (Bravecto®) to ten black bear cubs and collected blood samples 24 hours and 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70 days post-administration. Plasma was analyzed via high-pressure liquid chromatography (HPLC) with a method validated for black bears. Compartmental pharmacokinetic analysis was used to determine the primary parameters. This analysis showed that the maximum concentration (CMAX) at 1 day was 14.55 µg/mL (9.8 Std. Dev). The terminal half-life (T½) was 4.9 days (2.3 day Std.Dev), which is much shorter than measured in experimental dogs. These results will help to determine the effectiveness of fluralaner in black bears. Typically, 99.9% of the drug is eliminated in 10 half-lives. With this number, we can predict the length of time fluralaner may be detectable in the tissues, an important determination as black bears are considered game animals. This work represents specific information on a potential tool to be safely and effectively utilized in free-ranging black bears with sarcoptic mange.

# UNEXPECTED PARASITIC CYST LEADING TO DEATH OF A CRITICALLY ENDANGERED ROYAL BENGAL TIGER (PANTHERA TIGRIS TIGRIS) IN THE LAND OF GROSS NATIONAL HAPPINESS

Yoenten PHuentshok<sup>1</sup>, Kinley Choden<sup>2</sup>

<sup>1</sup>National Centre for Animal Health, <sup>2</sup>Nature Conservation Division

## Abstract

Tigers are listed as endangered species since 1969 but still their numbers and ranges have collapsed drastically in the last few decades. Bhutan is a global hotspot for wild felids. A population of about 103 adult tigers roam undisturbed in Bhutan with their habitats ranging from the southern foothills bordering India at 150 masl to alpine meadows and high Himalayas adjoining the Tibetean Autonomous region in the north at 4300 masl. On 21st March 2018, an adult male tiger roaming suspiciously close to human settlements in the capital of Bhutan was captured after videos and pictures of it wandering along the motor roads became viral on social media. The tiger was inappetent and showed nervous symptoms of in-coordinated gait, stumbling over objects, walking in clockwise circles with head held down, pressing its head on solid objects, being indifferent to the objects and people around and occasional fits-like attacks. Samples immediately tested and came out negative for rabies and canine distemper. Treatments didn't seem to work and the tiger died on 6th April 2018. On necropsy, two fluid filled cysts of approximately 1-2 centimeter diameter were were recovered from the brain of the tiger and PCR was used to amplify a small section of the cox1 and nad genes. Sequences from these PCR products: 330bp (cox1) and 466bp (nad) were used as input for BLAST. Preliminary characterization of an isolate from tiger matches sequences of Asian genotype of Taenia solium reported earlier from China, Indonesia and India. This is an interesting case of a human parasite leading to neurocysticercosis in a wild tiger. Is one unlucky adventurous individual or are there more tigers or other endangered wild carnivores suffering the same fate in the wilderness of Bhutanese forests?

## THE INFLUENCE OF EXPOSURE DOSE ON INFLUENZA INFECTION KINETICS IN MALLARDS

Susan Shriner<sup>1</sup>, Jeff Root<sup>1</sup>, Alan Franklin<sup>1</sup>, Kaci VanDalen<sup>2</sup>, Heather Sullivan<sup>1</sup>, Jeremy Ellis<sup>1</sup>, Mikaela Samsel<sup>1</sup>

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## Abstract

Influenza A viruses (IAVs) are endemic in wild birds, but can spillover into poultry and cause serious economic harm. Quantifying infection kinetics is critical to developing predictive disease models aimed at understanding pathogen spread in an effort to prevent spillover. In this study, we evaluated whether IAV exposure dose mediates infection dynamics in mallards, a common IAV reservoir host. We experimentally inoculated 3 groups of 10 mallards with 103, 104, or 105 EID50 of an H6N2 IAV collected from North American waterfowl during surveil-lance operations. Each inoculated mallard was housed with 3 naïve contacts. We collected fine-scale viral RNA shedding information throughout the infection in a scheme designed to capture the eclipse, exponential growth, and waning phases of infection. On the day of inoculation, we collected oral and cloacal swabs from all individuals every 2 hours post exposure, then every 4 hours the next day, every 8 hours for the following two days, and daily through day 10. All samples were tested by qPCR. We compared viral RNA output curves by assessing viral RNA peak load, total (cumulative) load, peak day, and shedding period for each dosage group.

In general, viral RNA shedding patterns varied across each of the metrics evaluated with significant individual heterogeneity evident across individuals. Specifically, for directly inoculated mallards 1) peak viral RNA loads increased with exposure dose (103.57 EID50 equivalents/mL for 103, 105.42 for 104, and 105.53 for 105), 2) total load increased with exposure dose (103.72 EID50 equivalents/mL for 103, 105.64 for 104, and 105.85 105), 3) peak day decreased with exposure dose (day 6 for 103, day 3 for 104, and day 2 for 105), and 4) the shedding period increased with exposure dose (4.54 days for 103, 6.23 days for 104, and 6.71 days for 105).

# ACTIVE AND PASSIVE DISEASE SURVEILLANCE IN WILD TURKEYS (MELEAGRIS GALLOPAVO) FROM PENNSYLVANIA, USA

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### Abstract

There are increasing concerns on the impacts of disease on wild turkeys (Meleagris gallopavo). Unfortunately, most regulatory agencies lack data on wild turkey disease/pathogens to address this concern. Toward that end, the Pennsylvania Game Commission enhanced active/targeted and passive surveillance efforts in wild turkeys beginning in 2013. During 2008-2018, 121 wild turkeys were submitted for necropsy; 93/121 (84%) were submitted during the enhanced surveillance period (2013-2018). Cases were examined to determine primary causes of morbidity/mortality through gross and microscopic examinations and ancillary tests. During 2013-2018, tissues and serum were collected from diagnostic cases and opportunistically from hunter-harvested turkeys. These samples were tested, as needed, for infection or exposure to specific pathogens. The most common causes of morbidity/mortality in the examined wild turkeys were avian pox (66/121; 54.5%), chronic dermatitis (15/121; 12.4%), and trauma (10/121; 8.3%), all of which were diagnosed throughout the entire study period (2008-2018). Additional diseases were diagnosed for the first time or more frequently during the enhanced surveillance period (2013-2018), including histomoniasis (7/121; 5.7%), lymphoproliferative disease (4/121; 3.3%), and infectious sinusitis (1/121; 0.8%). Skin lesions were the most common cause of submission (94/121; 77.7%) and were most often attributed to avian pox (66/94, 70.2%), chronic dermatitis (15/94; 16.0%), or lymphoproliferative disease (3/94; 3.2%). Through active/targeted surveillance, 75.3% (61/81) of wild turkeys were positive for lymphoproliferative disease virus, 61.9% (52/84) for Heterakis gallinarum, 28.6% (10/35) for Toxoplasma gondii, and 15.6% (15/32) for Borrelia burgdorferi. Antibodies to avian paramyxovirus-1 were present in 34.9% (22/63) of the wild turkeys, but none were seropositive to influenza A viruses (0%; 0/62). The presence of diseases and pathogens in wild turkeys in Pennsylvania are being defined through active and passive surveillance approaches. Such data can serve as the base to begin to address the broader questions of disease impacts on wild turkey populations.

## **EVALUATION OF POX VIRAL INFECTIONS IN CALIFORNIA HUMMINGBIRDS**

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<sup>1</sup>University of California, Davis, <sup>2</sup>San Francisco State University

# Abstract

The presence and impact of avian diseases on hummingbirds have been severely understudied despite their strong presence in California. Avian pox is caused by viral species in the genus Avipoxvirus. Avian pox can be transmitted by mosquitoes or a break in the skin barrier. This virus impacts the integumentary system and infected birds have classic wart-like lesions on the skin and keratin surfaces. To assess how avian pox is impacting hummingbirds in California, a quantitative polymerase chain reaction (qPCR) assay was developed. PCR analysis of blood samples, feather samples, and skin swabs of two hummingbird species (Anna's hummingbird [Calypte anna] and Allen's hummingbird [Selasphorus sasin]) were evaluated. Avian pox infections were detected using the qPCR assay. This study assessed if pox viral infections can be detected in different types of samples from hummingbirds, particularly feathers, and determined which species of Avipoxvirus are infecting hummingbirds. Ultimately, future research goals include estimating the prevalence of avian pox in these two hummingbird species and identifying possible vectors.

## **INVESTIGATING POECIVIRUS AS A CAUSE OF GLOBAL BEAK DEFORMITIES**

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<sup>1</sup>USGS Alaska Science Center, <sup>2</sup>University of California San Francisco

## Abstract

Avian keratin disorder (AKD), an emerging avian disease characterized by debilitating beak overgrowth, was first documented in Black-capped Chickadees (Poecile atricapillus) in Alaska in the late 1990s. Since then, AKD has been reported in more than 30 bird species worldwide. Among the recent observations are reports of AKD-like symptoms in Austral Thrushes in Argentina and endangered Red-cockaded Woodpeckers in South Carolina. Previous work has shown that a newly discovered virus, called Poecivirus, is strongly associated with clinical signs of AKD in Black-capped Chickadees in Alaska, and is suspected to be the etiological agent of this disease. Poecivirus has since been detected in deformed individuals of 8 different avian species (Black-capped Chickadee, Red-breasted Nuthatch, Northwestern Crow, Blackpoll Warbler, Mew Gull, Black-billed Magpie, Hairy Woodpecker, Red-tailed Hawk). However, the geographic scope and range of potential host species for this virus remains unknown. In this study, we used genetic techniques to test for the presence of Poecivirus in 17 species exhibiting AKD-like symptoms. Samples for this analysis were collected opportunistically from various locations in North America, South America, and Europe. Resultant viral sequences from these samples were used to assess host and geographical extent. Here, we present preliminary results of our efforts to sequence the full Poecivirus genome in avian host species from across the world.

#### **KEEP WILDLIFE WILD: HIGHLIGHTS OF A PUBLIC OUTREACH AND EDUCATION INITIATIVE IN WISCONSIN**

Mandy Kamps, Carissa Freeh, Dianne Robinson

Wisconsin Department of Natural Resources

# Abstract

Every year, wild animals are presented to wildlife rehabilitation centers because of unnecessary human interaction or are in illegal possession, despite education efforts by wildlife rehabilitators and natural resource agencies. In 2014, the Wisconsin Department of Natural Resources (DNR) began a Keep Wildlife Wild (KWW) initiative, in part due to public response related to illegally possessed wildlife cases. KWW objectives were to 1) put forth an unprecedented outreach and education effort to keep wild animals in the wild, 2) educate the public about wildlife natural history, and 3) reduce the number of illegally possessed wildlife cases. KWW accomplishments so far include: an electronic database to track illegally possessed wildlife cases, enhanced webpage information, mobile billboard, attendance at shows and fairs statewide, annual mailings to licensed wildlife rehabilitators, informational materials including situation guides, brochure, Project WILD lesson information, cartoon, bookmark, species-cards, social media posts/media appearances, 4-6th grade lesson plan, Keep Wildlife Wild week, and a 4-6th grade poster contest. The electronic database provides trend information on illegally possessed wildlife cases including locations, species, and resolution type. Results so far indicate the most common species include white-tailed deer (Odocoileus virginianus) and raccoon (Procyon lotor), and most common resolution is transfer to a rehabilitator. Since 2014, the KWW message has reached an estimated 3 million people. DNR staff indicate a perceived increase in public contacts related to baby wild animal questions however, the number of reported illegally possessed wildlife cases remain constant. KWW messages are reaching the public, but the consistent numbers of illegally possessed wildlife cases reported annually was unexpected. Perhaps more time is needed to see an effect, or different outreach methods are needed, but regardless, this information is useful for state agencies as a starting point when operating on a limited budget and educating the public to Keep Wildlife Wild.

# CONNECTION BETWEEN ENVIRONMENTAL CHANGE AND ZOONOTIC DISEASE RISK AT HUMAN-MACAQUE INTERFACES IN BANGLADESH: AN EXPLORATORY QUALITATIVE STUDY

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# Abstract

Many public health studies have been conducted that link environmental changes to emerging and re-emerging viruses. However, there has been limited research on the potential downstream effects of environmental changes on rhesus macaques (Macaca mulatta) and interactions with humans. This exploratory qualitative study aims to examine connections between perceived changes in macaque-human interactions as an effect of land-use change. Understanding these interactions is critical in the effort to understand interfaces with potential risk for viral disease spillover. Participant observations, ethnographic interviews (58), and focus group discussions (3) were conducted between August 2015- January 2016 in Old Dhaka, Madaripur, and Chandpur, Bangladesh. Participants reported a perceived that significant anthropogenic landscape transformations have led to increased human-macaque contact in study areas. All three sites reported having had substantial landscape alteration due to the construction of roads and buildings, commercial and residential, on open or agricultural land. Participants noted that the disappearance of forestland has increased macaques' dependence on fruit trees of household backyards. As natural water sources were depleted, from rivers and ponds being filled to support construction macaques were reported to become more dependent on human water sources. These changes have expanded their foraging areas and are now reportedly invading new areas where people are not culturally habituated to living with macaques. In response, many residents reported reacting aggressively, leading to biting and scratching events. However, many respondents accepted the presence of macaques at their homes though few consider macques as a threat for disease transmission. This study describes participants understanding of local environment changes, deforestation and the filling of water bodies, through construction and urban expansion has enabled increased macaque-human interactions in these study areas. Behavior change interventions should be developed to reduce zoonotic spillover by mitigating the risks associated with human-macaque interaction due to land use change.

# POLYCYCLIC AROMATIC HYDROCARBONS, HEAVY METAL POLLUTANTS, AND HEALTH IMPLICATIONS FOR THE AMERICAN MARTEN (MARTES AMERICANA) AND RIVER OTTER (LONTRA CANADENSIS) FROM ALBERTA, CANADA OIL SANDS

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<sup>1</sup>University of Guelph, <sup>2</sup>Environment and Climate Change Canada, <sup>3</sup>University of Ottawa

#### Abstract

The North American river otter (Lontra canadensis) and American marten (Martes americana) are economically valuable fur-bearing species in Canada and health indicators of their respective ecosystems. Polycyclic aromatic hydrocarbons (PAHs), present in bitumen, were produced at  $\sim$ 475 10<sup>3</sup>m<sup>3</sup>/day in the Alberta oilsands during 2018. PAHs are known carcinogens and mutagens, and toxicity can be exacerbated by heavy metal co-exposure. The health of wildlife in areas of oil sand operations is concerning; yet, little consideration has been given to the potential for PAH bioaccumulation and predisposition to disease. This study aimed to quantify PAHs and bioaccumulation of priority heavy metal pollutants in river otter and American marten samples from the Alberta oil sands; to screen samples for a subset of potential pathogens including: canine distemper virus (CDV), Toxoplasma gondii, Leptospira spp., and Dioctophyme renale; and to compare detected levels of contaminants, cortisol levels, and pathogen load across study sites. Tissue samples of 27 river otter and 30 marten were analysed for total recoverable trace elements using ICP-MS, for PAHs by GC-MS-MS, for T. gondii and Leptospira, and CDV in marten, by PCR. Kidneys were examined for D. renale. Hydrocarbon and trace element variables were modeled using logistic regression. Potentially pathogenic Leptospira spp. were detected in 83.3% (25/30) of marten and 96.3% (26/27) of otter; 53.5% (16/30) of marten samples tested positive for CDV, and D. renale was not detected in either species. Hydrocarbons had no effect on the incidence of disease detected in otter or marten; however, MeHg was a significant predictor of Leptospira in otter. Pb and Cu were significant predictors of CDV in marten. This study highlights the potential health impacts of oil and gas production to wildlife species. The data provided will contribute to wildlife management and environmental risk assessments in areas associated with oil sands.

# ISOLATION AND CHARACTERIZATION OF SKUNK ADENOVIRUS CAUSING SEVERE BRONCHOINTERSTITIAL PNEUMONIA IN REHABILITATED PORCUPINES IN NOVA SCOTIA, CANADA

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<sup>1</sup>Canadian Wildlife Health Cooperative, <sup>2</sup>Department of Fisheries and Oceans Canada, <sup>3</sup>Western College of Veterinary Medicine, <sup>4</sup>University of Guelph, <sup>5</sup>Hope for Wildlife Society

# Abstract

North American Porcupines (Erethizon dorsatum) are widely distributed across North America, ranging from the subarctic to northern Mexico. Despite the prevalence of this large rodent, there is relatively little information in the literature on porcupine diseases. We describe a previously unreported respiratory disease associated with skunk adenovirus infection in porcupines being treated at a wildlife rehabilitation facility in Nova Scotia, Canada. In 2017 and 2018, a total of 18 porcupines developed acute, severe respiratory signs after admission to the rehabilitation center. Of five porcupines submitted for necropsy, four had severe necrotizing bronchointerstitial pneumonia. Histologically, two of these porcupines demonstrated large intranuclear inclusion bodies in nasal and bronchial epithelium. Pan-adenoviral immunohistochemical staining performed on lung revealed adenoviral antigen in nuclei of bronchial epithelial cells and pneumocytes. Virus isolation using Vero cells was successful from two of the affected porcupines, and transmission electron microscopy demonstrated virus particles consistent with adenovirus. PCR and limited gene fragment sequencing showed 100% sequence identity to Skunk adenovirus PB1 (Genbank accession #KP238322.1) over 525 bp, and 99.78% sequence identity to Callithrix pygmaea adenovirus (accession #HM245776.1) over 460bp. This is the first report of viral pneumonia caused by skunk adenovirus in porcupines in North America. The initial source of the virus in these cases is not yet known, but this could represent an emerging disease in this species, with implications for post-rehabilitation release.

# USING EDNA TO DETECT BATRACHOCHYTRIUM DENDROBATIDIS IN YUKON, CANADA: IMPLICATIONS FOR AMPHIBIAN HEALTH

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## Abstract

Amphibian populations globally are under threat, and diseases are important factors contributing to regional declines. Chytridiomycosis, caused by the fungus Batrachochytrium dendrobatidis (Bd), has been associated with declines of some amphibian species and is a disease of concern. Bd was detected in Western Toads (Anaxyrus boreas) and Wood Frogs (Lithobates sylvaticus) in Yukon in 2008, and has been found in adjacent jurisdictions. While the effects of chytridiomycosis on amphibian populations in northern North America is poorly understood, investigation of Bd in amphibians at their northern range extent may provide important clues to the ecology of this pathogen and an opportunity to determine the geographic distribution of this potential threat. Environmental DNA (eDNA) is an emerging molecular approach for detection of genetic material sloughing off living organisms as well as detection of microbial communities in environmental samples. This methodology allows for non-invasive, cost-effective and sensitive pathogen surveillance. Water samples are collected, filtered, and the resulting filtrate is tested with validated molecular diagnostic methods. Prior to testing field collected samples, a validation study showed filters containing Bd genetic material should be preserved in ethanol and that dilution of the DNA extraction was necessary to obtain consistent results using real-time PCR. In June 2017, 28 water samples were collected from 14 different ponds and wetlands in southeastern Yukon for detection of genetic material from Bd. Bd was detected in both samples from one water body (2/28, 7.1% positive). The positive location has a known population of Western Toads, but is approximately 85 km west of the previous site of Bd detection in Yukon. This study demonstrates the value of using eDNA for wildlife pathogen surveillance, and confirms the presence of Bd in Yukon. Further studies are needed to determine the geographical extent of Bd and the possible impacts on Yukon's amphibians.

## PATHOLOGY OF CRYPTOSPORIDIOSIS IN RACCOONS (PROCYON LOTOR)

Viviana Gonzalez, Mauricia Navarro, Matthew Sheley, Francisco Uzal

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## Abstract

Cryptosporidiosis is a protozoal disease of public health importance, caused by Cryptosporidium spp., a group of apicomplexan parasites that cause intestinal disease and are found in ~150 mammal species, including humans. Cryptosporidium spp. appear to have a strong host-adaptation and thus wildlife infections do not appear to contribute to human disease. Nevertheless, some species such as Cryptosporidium parvum can be detected in numerous host species when a common source of infection exists (e.g. contaminated water). Studies describing the pathology and diagnosis of Cryptosporidium spp. infection in synantropic wildlife, such as the raccoon, are scant. We therefore characterized the pathology of cryptosporidiosis in two juvenile raccoons with history of progressive illness (# 1), and upper respiratory signs, hyporexia and weight loss (# 2), respectively. Grossly, both animals had pneumonia, and animal # 1 had also evidence of diarrhea. Microscopically, both animals had small intestinal crypt necrosis, villus atrophy and eosinophilia with intralesional Cryptosporidium spp., and pneumonia. In addition, animal # 2 had encephalitis. Intra-nuclear and intracytoplasmic inclusion bodies were observed in pulmonary bronchiolar epithelium and macrophages of both animals and in neurons of animal #2. Cryptosporidium sp. was detected by PCR targeting the 18S rRNA gene on mucosal scrapes of the small intestine of animal #1. The sequenced amplicon clustered with the Cryptosporidium skunk genotype. A retrospective search of the CAHFS database (1990-2019) produced eight raccoon cryptosporidiosis cases out of 318 raccoon submissions during that period. Seven of these cases were co-infected with canine distemper virus (CDV); it is likely that this virus increased susceptibility to cryptosporidiosis. This study has public health significance as raccoons co-habit frequently with humans.

# TRANSLOCATION OF GAMBEL'S QUAIL: IMPLEMENTATION OF WAFWA GUIDELINES FOR HEALTH SCREENING AND SAMPLING OF GALLIFORMS

Anne Justin-Allen, Callie Hartson

Arizona Game and Fish Department

#### Abstract

Gambel's quail (Callipepla gambelii) populations in much of Arizona have been heavily impacted in the last 5 years by severe drought conditions which have resulted in markedly reduced numbers of birds especially in areas without year-round water. Robust winter rains this year have created conditions which may support the recovery of quail populations. However with very few resident birds in some areas, this expansion is not likely to be significant. In light of this, the Arizona Game and Fish Department initiated a research project to examine translocation of Gambel's quail from abundant urban populations to native habitat in efforts to stimulate recovery of the depressed populations. The Western Association of Fish and Wildlife Agencies Wildlife Health Committee recently developed Guidelines for Health Screening and Sampling of Galliforms in order to reduce the risk of introducing diseases when translocating birds. We hypothesized that the disease profiles of bird from urban and native habitats would be different. Following the recommendations in the guidelines, we sampled source populations of Gambel's quail. We found that the urban populations were moderately parasitized by coccidia, giardia, and balantidium-like protozoa, and by trypanosome and hemoplasma organisms in the blood. Nineteen of 30 birds were seropositive for an avian adenovirus. No antibodies were detected to avian paramyxovirus or salmonella species; no adenovirus nucleotides were detected with PCR on oropharyngeal swabs. To reduce risk of introducing giardia and other parasites into the native populations, source birds were treated with ivermectin (~200mg/kg) and secnidazole (~50mg/kg). Tests on recipient populations are pending. There is very little information in the literature regarding diseases in western quail species and none regarding disease impacts on quail populations. It is important to recognize that moving wildlife without a clear understanding of the disease status of populations can jeopardize the success of the management actions.

# RE-EMERGENCE OF LEPTOSPIROSIS IN CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS) AFTER A FOUR-YEAR HIATUS: PATHOLOGIC FEATURES OF THE 2017-2018 EPIZOOTIC

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# Abstract

Leptospira interrogans serovar Pomona is enzootic in free-ranging California sea lions (Zalophus californianus;CSL) in northern California causing seasonal disease outbreaks with periodic large-scale epizootics. During these events, high rates of morbidity and mortality particularly among juvenile males, have been documented with nephritis as the most common necropsy finding.2,3Leptospirosis-related admissions to The Marine Mammal Center (Sausalito, CA) increased moderately during fall 2017 but beginning June 2018, there was a marked increase in cases presenting with clinical signs of renal insufficiency and failure (see Whitmer et al. this conference). Based on standard age-class criteria for this species, all four age classes were affected with the greatest prevalence of infection observed respectively among juveniles, followed by sub-adults, yearlings and adults. The epidemic curve for juveniles peaked in August while that for sub-adults peaked in October. Too few yearlings and adults were admitted to define disease characteristics in these, so an age-class specific pathology comparison was based on: (1) combined yearlings and juveniles and (2) combined sub-adults and adults. Group 1 had significantly (X2 8.225, p=0.0041) higher survival post admission than Group 2. At necropsy, lesions ranged in severity from nephritis with malnutrition and dehydration to an advanced uremic syndrome that included nephritis, buccal ulceration, hemorrhagic gastric ulceration, diffuse pulmonary edema, epidermal ulceration and occasional cerebral edema. For Class 1, approximately equal numbers of animals had these two pathologic presentations. In Class 2, however, significantly more animals presented with the advanced uremic syndrome (X25.451, p= 0.0196). The reason for this disparity is as yet unknown but may reflect a less competent immune response in younger animals with a fatal outcome earlier in the course of infection, or failure of older animals to strand and show clinical signs of illness until the disease is more advanced.

# PROTEOMIC ANALYSIS OF URINE FROM CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS) WITH AND WITHOUT LEPTOSPIROSIS

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## Abstract

Urinary markers for the assessment of kidney diseases in wild animals are limited, in part, due to the lack of urinary proteome data, especially for marine mammals. One of the most prevalent kidney diseases in marine mammals is caused by Leptospira interrogans, which is the second most common etiology linked to stranding of California sea lions (Zalophus californianus). We used proteomics to discover specific urine proteins as candidate markers of renal injury in California sea lions (CSLs: Zalophus californianus) with leptospirosis. This disease, caused by infection with pathogenic Leptospira spp., is one of the most common causes for CSL to strand. Infection is associated with renal disease ranging from subclinical to severe. Here, we analyzed urine proteins from CSLs divided into two groups: Leptospirosis CSLs (n=11) that stranded with active Leptospira infection with associated renal disease; Non-Leptospirosis CSLs (n=8) that were wild-caught, apparently healthy, and had no prior or current Leptospira infection. Tryptic peptides were separated by nanoflow chromatography and analyzed using a Thermo Orbitrap Fusion Lumos tribrid mass spectrometer. Spectra were searched against a combined Pacific walrus (Odobenus rosmarus divergens) and Weddell seal (Leptonychotes weddellii) proteome to make identifications. Experiment wide grouping resulted in identification of 2694 protein families, of which, 316 were differentially abundant (Benjamini and Hochberg corrected p < 0.05): 98 were increased and 218 decreased in the Leptospirosis group. Established tubular injury markers (e.g. Neutrophil Gelatinase lipocalin and Osteopontin) >15-fold elevated and nearly exclusively discovered in Leptospirosis. Inter-alpha-trypsin inhibitor heavy chain H1 showed the greatest positive fold change (35-fold) compared to CSLs without leptospirosis. Based on these results, analysis of urine proteins for the detection of kidney injury in CSLs offers promise for selection and validation of candidate biomarkers for identification of renal injury in marine mammals.

# INVESTIGATING THE DECLINE OF AFRICAN BUFFALO (SYNCERUS CAFFER) IN RUAHA NATIONAL PARK, TANZANIA

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## Abstract

The African buffalo (Syncerus caffer) population of Ruaha National Park, Tanzania has been declining over the last decades. Little was known about the health, movement, habitat use, and home ranges of the population, which is important for understanding possible reasons behind the decline. A total of 40 African buffalo were captured for health assessments, and of these, 12 cows from four different herds were collared with satellite transmitters. Data on home ranges, habitat use, and daily and seasonal movements were obtained from 11 animals over two-years. Across multiple herds in the park, bovine tuberculosis was detected with an overall prevalence of 22.5%, although no animals with obvious clinical signs were observed. Among buffaloes sampled from two large herds, exposure to Brucella abortusand Rift Valley fever virus was detected serologically with an overall prevalence of 22.5% and 27.5%, respectively, with two animals showing clinical signs of brucellosis (hygroma and orchitis). The home ranges of individual collared buffaloes ranged from 73 km2to 601 km2and were consistently larger in the wet season than in the dry season. Buffaloes strongly associated with habitats near the permanent water source of the Great Ruaha River in the dry season, and had little association to these sources in the wet season. With the exception of one buffalo, which appeared to be resident, all collared animals completed wet season migrations. The Great Ruaha River has experienced significant drying in the last decades due to water diversions upstream, which likely has reduced the suitable range for buffaloes as this species needs access to water daily. It is possible that loss of dry season habitat due to water scarcity has contributed to the population decline of the Ruaha buffaloes.

# UNEXPECTED DEATH OF A PUP IN THE BREEDING PROGRAM FOR THE CRITICALLY ENDANGERED ARCTIC FOX (VULPEX LAGOPUS) IN NORWAY

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## Abstract

In Fennoscandia, the arctic fox (Vulpes lagopus) is considered critically endangered. Management efforts in Fennoscandia have increased the population from less than 50 adult individuals in 2000 to above 300 in 2018. This increase is due in part to the success of a captive breeding program which has released on average 40 pups into the wild each year since 2006. Health management at the breeding station is therefore imperative to arctic fox survival.

In August 2018, a 3-4 month old pup was found dead at the breeding station. The head and neck was severely swollen. At necropsy profound subcutaneous edema and hyperaemia were observed. The facial and neck muscles were dark and swollen and some showed intramuscular haemorrhages. Severe edema was observed in the conjunctiva, nasal mucosa, lips, pharynx and larynx, the latter partially obstructing the airways. The thoracic cavity contained 20-30 ml serosanguinous fluid and there was moderate lung edema and congestion. Histologic findings included periacinar congestion in the liver, lung congestion and acute interstitial pneumonia with haemorrhages and fibrin in the alveolar lumens, and congestion in the kidneys and brain. Bacterial swabs from the chest cavity fluid resulted in sparse growth of Staphylococcus aureus and from the frozen liver growth of no known pathogens. The pup was otherwise in good body condition with no obvious traumatic lesions.

Although the cause of death is not clear, the findings are consistent with changes seen in dogs bitten in the snout, head or neck by the European viper (Vipera berus). Vipers are not known to be found at the same mountain elevation as the station; however, summer 2018 was unusually warm. Although a snakebite cannot be confirmed, changing climate and the potential for future warmer summers, highlights the need to consider new and different health threats for this critically endangered species.

# RAPID MOLECULAR MONITORING OF ARBOVIRUSES IN FIELD-CAUGHT ARTHROPODS USING UNIVERSAL PRIMERS

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## Abstract

The ongoing emergence of arthropod-borne viruses (arboviruses) poses major health concerns to all animals worldwide such as the epidemics caused by Zika and yellow fever viruses. The surveillance of arboviruses in field-collected mosquitoes may be an important tool for detecting emerging viruses; however, many resource-poor areas that are at the epicenter of wildlife-human interactions cannot afford routine surveillance technology. In response, we designed a relatively inexpensive, rapid, and sensitive molecular assay for arbovirus surveillance by developing an RT-PCR protocol with genera specific primers for the detection of the most common mosquito-borne alphaviruses, flaviviruses, and orthobunyaviruses. After validating our assay with 23 known viral stocks, we tested archived arthropods caught from 2009-2014 in the Southern Atlantic Forest in Bahía State, Brazil. We assayed 7699 macerated insects grouped into 304 pools and detected Alphavirus RNA in 22 pools, Flavivirus RNA in 17 pools and Orthobunyavirus in 2 pools. Our study confirmed the efficacy of primers used in infected mosquitoes and increased the detection of viruses from the genus Orthobunyavirus, which had not previously been tested with this protocol. We were particularly encouraged by the detection of viral RNA in crude preparations of naturally infected mosquitoes. Our results suggest that our RT-PCR assay may prove to be an important and inexpensive tool for the surveillance of the burden of arthropod borne arbovirus, especially useful in resource-poor areas. Furthermore, rapid detection of these viruses at the genus level could inform policy measures to address local priorities for vector control and disease management.

Anne Ballmann, Elizabeth Bohuski

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## Abstract

Fungal conidia of Pseudogymnoascus destructans (Pd), the causative agent of white-nose syndrome (WNS), have long been known to accumulate in the guano of infected bats, presumably from grooming activity during periods of arousal from winter hibernation. Guano has also proven to be a more sensitive sample source than skin swabs for detecting Pd on bats occupying Pd-contaminated hibernacula during summer months, and Pd DNA has been demonstrated in guano collected at above-ground summer roosts in areas where WNS occurs. However, the ability of Pd to survive transport through the gastrointestinal tracts of bats is not known. To address this question, an ~5 mm section of distal colon was sterilely collected from 26 bat carcasses stored frozen (-10° C) for up to 20 months at the USGS National Wildlife Health Center, including 17 bats diagnosed with WNS, six bats negative for Pd, and three bats not previously tested. Colonic contents from four species of bats (Myotis lucifugus, M. septentrionalis, Perimyotis subflavus, and Eptesicus fuscus) collected during winter hibernation (December to April) were evaluated. Quantitative PCR analyses targeting the intragenic spacer region of the Pd genome and fungal culture analyses were performed on all samples to test for the presence of Pd DNA and to assess the viability of the fungus, respectively. Pseudogymnoascus destructans was cultured from 13 of 19 (68.4%) colonic samples that tested positive for Pd by PCR. Greater relative abundance of Pd DNA in the samples was associated with positive fungal culture results, and length of storage time did not appear to negatively affect fungal viability. The ability of Pd to survive gut transit in bats has significant implications for the capacity of bats to move the fungal pathogen and disseminate it into new environments where the fungus can persist.

# NEXT GENERATION SEROLOGY: INTEGRATING CROSS-SECTIONAL AND CAPTURE-RECAPTURE APPROACHES TO INFER DISEASE DYNAMICS

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## Abstract

Predicting the epidemiological dynamics of a pathogen requires a quantitative understanding of its circulation in its host populations. However, data collection is often limited by logistical and cost constrains, notably in wild populations, sometimes impacting the capacity to infer epidemiological dynamics, especially when indirect infection data, such as serological data, are used. It is thus important to optimize field sampling designs by accounting for both the constrains and the quality of the inference. In this context, we first compare the efficiency of two approaches classically used in disease ecology to estimate the force of infection (i.e., the rate at which susceptible individuals become infected) from serological data: cross-sectional sampling from unmarked individuals and longitudinal capture-recapture setups, which generally involve more limited numbers of marked individuals due to cost and logistical constrains. Then, inspired from tools developed in the field of population ecology, we explore the benefits of integrating both cross-sectional (seroprevalences) and longitudinal (individuals histories) datasets. To do so, we use a simulation approach combined with empirical data collected in three colonial vertebrate populations: gulls exposed to the parasite Toxoplasma gondii, albatrosses exposed to the bacteria Pasteurella multocida and bats exposed to a rabies virus. Our results highlight that key elements to determine optimal sampling designs are: host species life history, the temporal persistence of the antibody response and the degree of a priori knowledge and uncertainty on demographic and epidemiologic parameters. In the case of long-lived species exposed to infectious agents resulting in persistent antibody responses, integrated designs are especially valuable. Overall, we argue that serology studies could greatly benefit from the opportunity of integrating cross-sectional and longitudinal designs and propose a statistical method to do so.

### MYCOPLASMA SPP. DISCOVERIES IN ALASKAN WILDLIFE

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## Abstract

Multiple species of bacterial genus Mycoplasma are respiratory-associated pathogens that can contribute to human and animal disease. One example is Mycoplasma ovipneumoniae, a pathogen associated with multifactorial and typically polymicrobial respiratory disease in members of the subfamily Caprinae. This bacterium has been reported in high morbidity and mortality pneumonia outbreaks in bighorn sheep, muskoxen (Ovibos moschatus) pneumonia outbreaks, and recently in association with bronchopneumonia in mountain goat (Oreamnos americanus) kids. Lack of both detection of this bacterium and mortality events in Dall's sheep or other free-ranging Alaskan wildlife, led to the misconception that this bacterium was not present in Alaska. In order to characterize respiratory pathogens, including Mycoplasma spp., in Alaskan wildlife, nasal swabs and tissue samples were collected 2012-2017. We detected M. ovipneumoniae in Dall's sheep (Ovis dalli dalli), mountain goats, and non-Caprinae species previously believed to be unable to carry the pathogen including moose (Alces alces gigas) and caribou (Rangifer tarandus granti). Although no direct evidence linking respiratory disease and M. ovipneumoniae has been found in Alaskan wildlife, we have detected M. ovipneumoniae in lung tissue of a caribou calf that died of polymicrobial bronchopneumonia. A larger survey of hunter-harvested and live-capture released ungulate species in 2018 sought to determine host species and geographic distribution of exposure to this potential pathogen. In addition to M. ovipneumoniae, we describe detection of other Mycoplasma spp, including a previously uncharacterized mycoplasma. This organism has been detected primarily in moose, domestic sheep and domestic goats, but also other species at much lower rates of detection including caribou, mountain goats, and Dall's sheep. Because of potential population impacts of Mycoplasma spp., it is critical to further understand the distribution, prevalence, and potential adverse health (individual and population level) impacts of M. ovipneumoniae and other Mycoplasma spp. in Alaskan wildlife and domestic small ruminants.

# RAPID DNA DETECTION AND ENHANCED GROWTH OF TUBERCULOSIS FROM RESPIRATORY AND TISSUE SAMPLES OBTAINED FROM AFRICAN RHINOCEROS (DICEROS BICORNIS, CERATOTHERIUM SIMUM) AND AFRICAN ELEPHANTS (LOXODONTA AFRICANA)

Wynand Goosen<sup>1</sup>, Josephine Chileshe<sup>2</sup>, Candice de Waal<sup>2</sup>, Tanya Kerr<sup>2</sup>, Peter Buss<sup>3</sup>, Paul van Helden<sup>2</sup>, Sven Parsons<sup>2</sup>, Michele Miller<sup>2</sup>

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# Abstract

Background: While some TB-infected (M. bovis and M. tuberculosis) rhinoceros and elephants may develop clinical signs, most are asymptomatic, but more importantly, still able to shed TB. Therefore, undiagnosed animals shedding could possibly put other animals and humans at risk of infection. This highlights the need for 1) a sensitive rapid point-of-care diagnostic test for TB infection in these animals and 2) an enhanced mycobacterial culturing method able to detect low levels of TB in ante-mortem respiratory samples full of rapid growing environmental mycobacteria. Hypothesis: TB DNA can be successfully detected and its growth enhanced from rhinoceros and elephant respiratory and tissue samples. Materials and Methods: Banked, opportunistically obtained respiratory- and tissue samples from n=50 free-ranging African rhinoceros and elephants samples were used for this study. For the direct detection of TB DNA, the human GeneXpert MTB/RIF Ultra qPCR assay was used and the new TiKa-MGIT system for culturing and compared to the conventionally used Mycobacterium BACTEC 960 MGIT system. Results: The commercially available human nucleic acid amplification test, GeneXpert MTB/RIF Ultra assay, detected (19/20) known TB-infected samples and no uninfected samples (0/30). Compared to the conventional MGIT system (6/20), the new TiKa-MGIT culturing system detected the most (13/20) known TB-infected samples, with a significant lower false-positivity rate (7/30 versus 29/30) and average time to positivity (6 days versus 14 days). Conclusions: The GeneXpert MTB/RIF Ultra assay can rapidly detect TB DNA from African elephant and rhinoceros respiratory and tissue samples. The new TiKa-MGIT culturing system significantly enhances the growth of TB from tissue samples and respiratory samples full of rapid growing environmental mycobacteria. Originality: This is the first report for using these molecular tools on wildlife samples. Significance: These findings will benefit veterinarians, managers and policy makers working in zoos and range countries. Results will facilitate TB disease surveillance.

# DISEASE SURVEILLANCE DURING THE FIRST YEARS OF THE REINTRODUCTION OF AN APEX PREDATOR IN SOUTHWESTERN SPAIN

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#### Abstract

Efforts to restore Iberian lynx (Lynx pardinus) populations in Extremadura (Southwestern Spain) have been carried out since 2014. One of the measures to ensure the success of this program is to examine the effect that diseases may have on reintroduction. Because diseases may be highly located at certain sites as a result of the specific ecological requirements of pathogens and/or vectors, reintroduced individuals may present risk of infection once released. To determine which pathogens the reintroduced individuals may encounter, we performed a molecular and sero-epidemiological surveillance in reintroduced and wild born lynxes. During the first five years of the reintroduction, 36 captive-bred lynxes were reintroduced and 45 were wild born. Samples were obtained from 22 reintroduced lynxes and 10 wild individuals. Serum samples were tested against feline calicivirus, feline herpesvirus, feline parvovirus, feline leukemia virus, feline immunodeficiency virus and canine distemper virus. Blood samples were also analyzed by PCR for feline leukemia provirus, feline parvovirus, feline coronavirus, feline herpesvirus, feline calicivirus, canine distemper virus, Leptospira spp., andCytauxzoon spp. We also screened for suid herpesvirus 1 in dead lynxes. Additionally, whenever possible, we investigated other causes of disease in necropsies. In four out of eleven post mortem examinations, we found active infection of Cytauxzoon spp. and Aeromonas veronii (same lynx); feline leukemia provirus and suid herpesvirus 1 (same lynx), feline parvovirus, and a Streptoccocus canis necrotiziting myositis. Evidence of exposure to feline parvovirus, feline herpesvirus, feline calicivirus and feline coronavirus were observed in 3/31;6/31; 6/31; 1/31 of the lynxes respectively. In such small, endangered population we recommend continuing a disease surveillance program to determine prognostic factors of survival, understand the role that disease may play during the reintroduction and anticipate disease outbreaks which may pose a risk for the entire reintroduced population.
# ANALYZING THE DYNAMICS OF AUJESZKY'S DISEASE VIRUS INFECTION IN WILD BOAR (SUS SCROFA) IN ENZOOTIC SCENARIOS

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# Abstract

Aujeszky's disease virus (ADV) virus is enzootic in Iberian wild boar, posing a threat for the eradication of Aujeszky's disease in extensive domestic pigs. Understanding the dynamics and drivers of ADV infection in wild boar will help preventing viral transmission at the wild boar-pig interface. This study seeks to describe the dynamics of ADV infection in enzootic wild boar populations and identify drivers of ADV infection dynamics. Wild boar sera and oropharyngeal tonsils (TN) collected along 11 years in 3 hunting estates in Spain were tested for ADV antibodies and DNA, respectively. We tested the hypotheses that population immunity modulates ADV infection risk (H1), and that detecting ADV in TN is a good proxy of ADV infection pressure (H2). We built up logistic regression models to test the influence of host population and host individual factors on the risk of new ADV infections and on the presence of ADV in TN. According to H1 we expected a negative association between the proportion of ADV antibody positive wild boar in a given year and the risk of new ADV infections. However, the association was positive instead, so H1 was rejected. If detecting ADV in TN was a good indicator of ADV infection pressure, a positive association with ADV antibody positive wild boar would be expected according to H1. However, this was not observed and H2 was also rejected. Nevertheless, we confirmed that ADV infection in enzootic scenarios is a dynamic but potentially predictable phenomenon. The risk of ADV infection changes between consecutive years and changes are positively associated with the proportion of infected wild boar in the population. These results would be useful to design ADV control strategies in wild boar to prevent transmission at the wildlife-livestock interface and maintain the free ADV status of pigs in Spain.

# TRACKING AN INVADER: WILDLIFE SURVEILLANCE FOR HAEMAPHYSALIS LONGICORNIS IN THE EASTERN U.S.

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## Abstract

Haemaphysalis longicornis, also known as the Asian longhorned tick, is native to eastern Asia, but it has become invasive in several countries including, Australia, New Zealand, and now the U.S. Since its 2017 discovery in New Jersey, the presence of H. longicornis has been confirmed in 9 additional states in the eastern U.S., on a variety of domestic animals, wild and feral carnivores, cervids, rodents, and avian species. Archived specimens previously identified as H. leporispalustris, the native rabbit tick, were recently re-examined and determined to be the first detection of H. longicornis on free-ranging wildlife in the U.S. (white-tailed deer [WTD] in 2010). To address misidentification concerns, we developed a restriction fragment length polymorphism (RFLP) assay that can quickly and accurately distinguish between morphologically similar native and exotic Haemaphysalis species. To better assess the current geographic distribution of H. longicornis and to identify wild mammal and avian host species, we conducted active wildlife surveillance at two sites in New Jersey and Virginia with known infestations. In addition, we conducted a passive regional survey in collaboration with wildlife biologists and rehabilitation centers in the southeastern U.S. For rehabilitation centers, all wildlife species were sampled, whereas regional surveillance targeted cervids and bears. Collectively, our surveillance detected H. longicornis infestations on 16 individual cervids (WTD and elk) from six states, 50 mesomammals (raccoon, Virginia opossum, striped skunk, red fox, and gray fox) from three states, 2 coyotes, 4 woodchucks, 1 eastern cottontail, and 1 red-tailed hawk, from one state each. This surveillance effort resulted in numerous new host, state, and county records for H. longicornis. Although infestation prevalence was high for several wildlife species, the availability of cervid samples (specifically WTD), through vehicle strike, rehabilitation, depredation removals, and seasonal hunting, suggests they are potentially efficient sentinels to determine geographic distribution of H. longicornis.

#### CHARACTERIZING LANDSCAPE FACTORS OF TWO WEST NILE VIRUS VECTORS IN PENNSYLVANIA, USA

Dominika Dec Peevey<sup>1</sup>, W. David Walter<sup>2</sup>, Lisa Williams<sup>3</sup>, Andrew Kyle<sup>4</sup>

<sup>1</sup>Pennsylvania State University, <sup>2</sup>USGS Pennsylvania Cooperative, <sup>3</sup>Pennsylvania Game Commission, <sup>4</sup>Department of Environmental Protection

#### Abstract

Zoonotic diseases have increased global concern and increased human illnesses with viral outbreaks such as yellow fever, dengue fever, West Nile and Zika virus. Emerging and established zoonotic diseases are being studied by the World Health Organization and the Center for Disease Control with prime examples being West Nile virus (WNV) and diseases spread by mosquito or other insect vectors. Tracking the spread of WNV is a crucial step in predicting and controlling outbreaks in wildlife that can potentially lead to zoonotic spillover. The focus of this study was to compare WNV prevalence and of Culex restuans and Culex pipiens across urban and rural used in Pennsylvania. These two WNV vectors are implicated in WNV outbreaks in birds, non-domestic mammals, and, in some cases, humans. The Pennsylvania Department of Environmental Protection has collected mosquitoes statewide across Pennsylvania. Mosquito samples were routinely collected during prime mosquito activity months (May to October) from 2001 to 2017. Each mosquito sample was speciated, counted, pooled into sub-samples, and tested for West Nile virus. Preliminary results indicate that Culex restuans and Culex pipiens across the Pennsylvania landscape. This research analyzes which landscape (i.e. urban or rural) is most at risk for enzootic and potential human epidemics, from which vector species, and how variation in environmental characteristics could affect these vector populations.

# A BITTER PILL TO SWALLOW - EXAMINING ANTIBIOTIC RESISTANCE IN SENTINEL MARINE MAMMALS OF THE SALISH SEA

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#### Abstract

Antibiotic resistance is documented in multiple marine species. Data on resistance in marine species in Washington State's inland waters, known as the Salish Sea, are relatively limited. Preliminary work reported resistance in young harbor seals (Phoca vitulina) in rehabilitation and in breath from local southern resident killer whales (Orcinus orca), as well as anecdotal reports from stranding necropsies. A larger dataset with multiple species, age classes, and locales is warranted to determine if resistant bacteria exhibit patterns within this urban marine ecosystem, and if they pose a threat to other marine mammal and human health. This crowdfunded and Wildlife Disease Association-supported project sought to: detect and describe the presence and distribution of antibiotic resistance in two key Salish Sea marine mammal species; determine differences in resistance between harbor seals and harbor porpoises (Phocoena phocoena); and document geographic patterns. These species were selected because they are abundant and relatively site-specific within the Salish Sea. A cross-sectional study was conducted to calculate prevalence (%) of resistant bacteria from 69 dead-stranded harbor seals and 69 porpoise, of all age classes and sexes. The study area was divided into northern and southern sampling regions. We hypothesized that resistance prevalence would: be < 5% overall, differ between harbor seals and porpoises, and between the two sampling regions. Sterile swabs of the colon and lesions noted on necropsy were submitted for aerobic culture and sensitivity to a suite of 16 commonly used antibiotics using standard laboratory techniques. Preliminary results to-date indicate resistance in both species (total sampled(n); % with any resistance; % with multi-drug resistance): seals (n=16; 69%; 38%) and porpoises (n=8; 63%; 63%) all age classes and sexes represented. Genotyping and sequencing of E. coli cultures are ongoing. Study results will better define antibiotic resistance prevalence patterns and risk factors in Salish Sea marine mammals.

# MANAGING RETICULOENDOTHELIOSIS VIRUS IN A CAPTIVE BREEDING AND RELEASE PROGRAM FOR ENDANGERED ATTWATER'S PRAIRIE-CHICKENS (TYMPANUCHUS CUPIDO ATTWATERI)

Holly Haefele<sup>1</sup>, Pam Ferro<sup>2</sup>, Joseph Flanagan<sup>3</sup>, Kristina Borgstrom<sup>1</sup>, Michael Morrow<sup>4</sup>

<sup>1</sup>Fossil Rim Wildlife Center, <sup>2</sup>Texas A&M University Medical Diagnostic Laboratory, <sup>3</sup>Houston Zoo, <sup>4</sup>Attwater Prairie Chicken National Wildlife Refuge

# Abstract

The endangered Attwater's prairie-chicken (APC; Tympanuchus cupido attwateri) has been reared in captivity for release into historical habitat since 1992. Despite continued improvement in captive rearing, reticuloendotheliosis virus (REV) continues to hinder program success. REV is a gammaretrovirus associated with neoplasia and immunosuppression in poultry. Infection in captive APCs has resulted in mortality and neoplasia, mainly lymphosarcoma. Managing REV in APCs poses medical and financial challenges; outbreaks have significantly impacted genetic variability and the number of APCs available for release. Several new methods were tested to mitigate the impact of REV, including breeding REV positive birds and improved testing strategies. In an effort to better understand REV pathogenesis and transmission, 11 REV PCR positive hens were bred and produced chicks for possible release. Vertical transmission is rare in poultry, and was not observed in APCs. The number of eggs produced per hen, the fertility/egg viability, and chick survival were not significantly different between REV PCR positive and negative hens. However, PCR positive hens were less productive, as hatchability of their eggs was significantly lower. Routine testing allows for early detection of infected birds. Historically, only whole blood PCR or real-time PCR (provirus detection) was used for antemortem diagnosis and screening. Therefore, to perform the necessary weekly testing during outbreaks, birds underwent the stress and trauma of frequent handling. Recently, a real-time RT-PCR assay was designed to detect virus in feces, which has several advantages: samples can be collected with minimal stress, samples can be pooled to reduce testing costs, and the assay detects shed virus. Due to the epizootic nature of REV in APCs, it is too early to definitively determine the impacts of fecal testing on reducing the number and severity of outbreaks, however, improved understanding and testing are vital for a species still dependent on captive breeding.

# POST-CAPTURE MORTALITIES IN ROCKY MOUNTAIN MULE DEER ASSOCIATED WITH TRAUMATIC COCCYGEAL INJURIES RESULTING FROM HELICOPTER NETGUN CAPTURE: A TAIL OF TWO DEER

Nicholas Shirkey<sup>1</sup>, Brandon Munk<sup>1</sup>, Jane McKeever<sup>2</sup>, Timothy Taylor<sup>2</sup>, Mike Morrison<sup>2</sup>, Tom Stephenson<sup>2</sup>

<sup>1</sup>California Department of Fish and Wildlife, Wildlife Investigations, <sup>2</sup>California Department of Fish and Wildlife, Inland Deserts Region

# Abstract

Helicopter netgun capture is considered one of the most safe, efficient, and effective means of capturing large numbers of free ranging mule deer for management and research purposes. However, it is not without risk to both capture personnel and the animal. The California Department of Fish and Wildlife (CDFW) has had a longterm deer population study supported by helicopter netgun captures in winter range habitats of the Eastern Sierra Nevada. Between 2014-2018, we captured 604 adult female Rocky Mountain mule deer (Odocoileus hemionus hemionus) in sagebrush scrub and Pinyon-Juniper wintering habitat of Inyo and Mono counties, CA and in Mineral, Lyon and Douglas counties, NV. These captures provide a large, multi-year dataset to assess relative risk factors associated with capture related mortalities both at the time of capture and up to 15 days post capture. Risk factors associated with post-capture mortality were determined by assessing a suite of capture related variables using logistic regression. Major injuries sustained at the time of capture, particularly sacral and caudal vertebral injuries, were a significant risk factor for post-capture mortality. To the best of our knowledge, such sacral and caudal vertebral injuries have never been reported in association with helicopter net-gun captures, and highlight the importance of a good physical exam. These coccygeal injuries were first noted because of decreased tail and anal tones associated with palpable crepitus, dislocation, or fracture on digital rectal examination. We believe that the prevalence of this type of injury and likely other identifiable injuries that have yet to be elucidated, may be underreported during netgun captures, particularly when captures are performed in rocky terrain with little to no snow cover. For managers, biologists, and veterinarians seeking to better understand and mitigate capture-related mortalities, it is important to first define what those mortality factors are.

# GENOTYPES OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IN TICKS FROM WILD UNGULATES IN SPAIN

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## Abstract

Crimean-Congo hemorrhagic fever virus (CCHFv) is the causative agent of the severe tick-borne, often fatal, zoonotic Crimean-Congo hemorrhagic fever (CCHF), which is widely distributed worldwide. The CCHFv transmission to humans occurs through tick bite, crushing of engorged ticks, or by contact with infected host blood. Previously, CCHFv genotype Africa III was reported in Spain. Given the emergence of CCHF and the role of ticks in pathogen maintenance and transmission, we investigated the presence and genotype identity of the virus in tick species parasitizing abundant wild host species in southwestern Spain. A total of 613 ticks were collected from hunter-harvested wild ungulates in twenty locations throughout southwestern Spain. Ticks were identified, RNA was extracted and analyzed by a nested RT-PCR targeting CCHFv S segment, and the amplicons were sequenced. Presence of CCHFv human genotype Europe V was detected in Hyalomma lusitanicum and Dermacentor marginatus ticks collected from red deer, fallow deer and Eurasian wild boar in different locations from southwestern Spain. The presence of CCHFv in different tick species collected from various wild host species and localities provided strong evidence of widespread CCHFv presence in the region, suggesting that CCHFv circulation in Spain requires more attention. Additionally, the identification of the CCHFv genotype Europe V in ticks suggested its presumably introduction in Spain from Eastern Europe.

# HUNTING PRESSURES SURROUNDING MYANMAR'S NORTH ZAMARI KEY BIODIVERSITY AREA: OPPORTUNITIES AND CHALLENGES FOR WILDLIFE CONSERVATION AND PUBLIC HEALTH IN THE BAGO YOMA

Tierra Evans<sup>1</sup>, Theingi Win Myat<sup>2</sup>, Pyaephyo Aung<sup>3</sup>, Min Thein Maw<sup>4</sup>, Zaw Min Oo<sup>5</sup>, Aung Than Toe<sup>1</sup>, Tin Htun Aung<sup>3</sup>, Nang Sarm Hom<sup>2</sup>, Khin Thawda Shein<sup>5</sup>, Kyaw Zin Thant<sup>2</sup>, Kirsten Gilardi<sup>1</sup>, Peter Barry<sup>6</sup>, Hlaing Myat Thu<sup>2</sup>, Christine K. Johnson<sup>1</sup>

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# Abstract

Wildlife hunting and trade is a leading threat to biodiversity conservation and a global driver of the spread of infectious diseases. The dynamics of these practices have received few quantitative assessments in Myanmar, a country considered to be the last frontier of biodiversity in Asia. In this study, as part of an investigation of the epidemiology of zoonotic viruses in forest communities, focus group discussions, questionnaires (n=161), and hunter follows were conducted to better understand patterns of hunting and trade near the North Zamari Key Biodiversity area. Fifty percent (80/161) of study participants reported having hunted a wild animal and 67% (106/161) reported having slaughtered a wild animal. Wildlife hunting was associated with being male (P<0.001), being a protected area worker (P<0.001) and being an extractive industry worker (P<0.001). Thirty-four species of wild animals were reported as hunted, including several species designated as critically endangered (Manis javanica), endangered (Panthera tigris, Manouria emys, Indotestudo elongate, Trachypithecus phayrei) and vulnerable (Python bivittatus, Ophiophagus hannah, Rusa unicolor, Macaca leonina, Ursus thibetanus, Helarctos malayanus, Prionailurus viverrinus, Arctictis binturong, Panthera pardus). Phayre's langur's were the most frequently hunted species, raising concern of human exposure to zoonotic diseases of non-human primate origin. Half of the species (17/34) reported as hunted had local medicinal and/or trade value in Myanmar and China. Species with a medicinal use were 24 times more likely to be categorized as threatened compared to species without a medicinal use (OR=24, P= <0.001). Eighty-two percent (28/34) of the species traded were traded locally, with the remainder destined for China. Identifying bush-meat's value, as a dietary staple, medicinal source, as well as a trade commodity is vital for targeting future conservation interventions and, equally important, for predicting the impacts of wildlife declines and the potential spread of zoonotic diseases on human livelihoods.

# INFECTIOUSNESS OF THE NATIVE RODENTS DEGU AND DARWIN'S PERICOTE IN A TRYPANOSOMA CRUZI WILD VECTOR IN SEMIARID CHILE

Juana Paola Correa, Nicol Quiroga, Antonella Bacigalupo, Andrea Yañez-Meza, Esteban San Juan, Sophie de Bona, Rodrigo García de Cortázar, Carezza Botto-Mahan

Universidad de Chile

# Abstract

Trypanosoma cruzi, a protozoon infecting several hosts including mammals and hematophagous triatomine vectors, causes Chagas disease in humans. Host competence of mammals is expected to be a species-specific trait, and it can be studied measuring host infectiousness, defined as the proportion of vectors that one host species can infect when the insect vectors feed on them. In semiarid Chile, where the main wild vector of T. cruzi is the endemic Mepraia spinolai, two native rodent species, the diurnal gregarious Degu (Octodon degus, Octodontidae), and the nocturnal solitary Darwin's Pericote (Phyllotis darwini, Cricetidae) are abundant, widely distributed and infected by T. cruzi. Our objective was to evaluate the infectiousness of these two rodent species towards M. spinolai. In the summer of 2018, 22 degus and 32 Darwin's pericotes were captured from natural populations and, under anesthesia, submitted to xenodiagnosis procedure using six uninfected first instar nymphs from laboratory-reared colonies. Extracted DNA from mammals' blood samples and abdominal content of triatomines was submitted to real-time PCR targeting a conserved region of nuclear DNA of T. cruzi. Infection frequency was 27.3% in degus and 81.3% in Darwin's pericotes, while overall infectiousness was 69.9% and 63.0% in each species, respectively, without differences between them (p=0.572). All rodent individuals were capable to infect at least one of the nymphs exposed. Our preliminary result indicates that even when these two species have strong behavioral (e.g., activity time and social structure) and evolutionary differences, both have similar capability to infect their cohabiting triatomines. Probably this lack of differences responds to ecological reasons, i.e., both species live in sympatry with M. spinolai, and both are suitable blood sources to this opportunistic vector species. Funding FONDECYT 1170367 and 11181182.

# UNUSUAL PRESENTATION OF PERICARDITIS SECONDARY TO DISSEMINATED COCCIDIOIDOMYCOSIS IN A STRANDED SOUTHERN SEA OTTER (ENHYDRA LUTRIS NEREIS) FROM CENTRAL CALIFORNIA

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## Abstract

A fresh dead, emaciated subadult female southern sea otter (Enhydra lutris nereis) was recovered within Morro Bay, California in September, 2016. Grossly, the pericardial sac was diffusely opaque, palpably thickened, white, and massively enlarged, filling approximately one third of the chest cavity. The massively dilated pericardial sac contained approximately 100 ml of yellow/ tan, slightly opaque, viscous fluid admixed with many hundreds of firm, 0.5 to 3 cm diameter, free-floating, single to multinodular masses. On cut surface the masses were composed of closely packed, homogenous-looking, light pink, pale yellow or white opaque spheres. Histologically, the spheres were composed of homogenous to finely fibrillar, PAS-positive pale eosinophilic material (fibrin), separated by small clear spaces. Low numbers of 20 to 50 µm diameter round, thick-walled structures with a thin, light gold refractile outer wall, and a basophilic, granular-appearing interior (fungal spherules) were present within and adjacent to masses sampled nearest to the epicardial surface, along with sparse inflammatory cells. A few spherules contained aggregates of 1-3 µm diameter round basophilic to pale eosinophilic structures (endospores). The suspected fungal spherules and endospores were PAS- and GMS-positive. Culture of pericardial fluid revealed low growth of Coccidioides immitis/ posadasii, and very low growth of E. coli and Corynebacterium sp. Disseminated coccidioidomycosis caused by Coccidioides sp., likely C. immitis was confirmed via histopathology and fungal culture; PCR characterization of the fungal isolate is planned for species confirmation. Although mild to moderate pericardial or epicardial fibrin exudation has been reported previously from Coccidioides sp.-infected humans and animals, massive distension of the pericardial sac by large, free-floating multinodular fibrin spheres has not previously been described to our knowledge. A substantial increase in clinically significant or fatal Coccidioides infections of humans, terrestrial animals and sea otters has been observed in San Luis Obispo County since 2016.

# CONTROL OF TICK INFESTATIONS IN WILD ROE DEER (CAPREOLUS CAPREOLUS) VACCINATED WITH THE Q38 SUBOLESIN/AKIRIN CHIMERA

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## Abstract

Ticks (Acari: Ixodidae) transmit a wide variety of pathogens to vertebrates including viruses, bacteria, protozoa and helminths. Ticks are considered to be the most important vectors of disease-causing pathogens in domestic and wild animals, and emerging and re-emerging tick-borne diseases (TBD) exert an enormous impact on them. Wild ungulates are hosts for different tick species and tick-borne pathogens that affect human and animal health, playing an important role in their transmission and maintenance. Consequently, the control of tick infestations and tick-borne pathogens prevalence is essential in some regions. Acaricides and animal management or culling have been used for the control of tick infestations and TBD, but tick vaccines constitute the best alternative to reduce the impact of acaricides on tick resistance and the environment. Previous results of controlled vaccine trials have shown that the Q38 Subolesin/Akirin chimera containing conserved protective epitopes is a good candidate for the control of tick vector infestations. However, vaccination trials are necessary to validate these results under field conditions.

In this study, we characterized the effect of Q38 vaccine on a wild population of European roe deer (Capreolus capreolus) in the Andalusian roe deer Reference Station (Junta de Andalucía, Cádiz, Spain). In this location, roe deer suffer especially severe parasitic conditions in some periods and commercial pesticides and ixodicides that are authorized to control ticks without specificity are frequently applied in the field, posing a threat to the environment. Animals vaccinated over a three years period showed an antibody response to vaccine antigen and a reduction in tick infestations when compared to untreated controls. These results provided evidence supporting the efficacy of Q38 vaccine for the control of tick infestations in wild hosts under field conditions.

## **MYXOMA VIRUS IN TICKS FROM LEPORIDS, SPAIN, 2018**

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# Abstract

Myxoma virus (MV) causes important rates of morbidity and mortality in leporids. MV specific DNA was detected 47.7% of 67 tick pools (nymphs and adults of Rhipicephalus pusillus, and larvae and nymphs of Hyalomma lusitanicum) feeding on rabbits, and 100% in 2 adults of R. pusillus collected from hares in Southern Spain. Partial sequence of the viral major envelope protein gene showed a mutation (G383A) within the MYXV\_gp026 locus between the rabbit strain and hare strain, not observed before in Spain. Different virus may be the cause of the high mortality rate in hares. Viral DNA presence in ticks is detected for the first time, and the role of ticks as competent vectors still need to be elucidated.

# PHYLOGENOMIC CHARACTERIZATION OF NOVEL ORBIVIRUSES ISOLATED FROM WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS)

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## Abstract

The genus Orbivirus within the family Reoviridae includes arboviruses with non-enveloped nucleocapsids, composed of outer and inner proteineacous layers that surrounds a segmented, dsRNA genome. Orbiviruses infect a wide range of mammalian species and are transmitted by ticks, mosquitoes, gnats, and midges. In 2017, samples from moribund white-tailed deer were submitted to the University of Florida Cervidae Health Research Initiative (CHeRI) for diagnostic evaluation. Aliquots of homogenized spleen specimens were inoculated onto Aedes albopictus clone C6/36 monolayers resulting in cytopathic effects. The viral genomic RNA was extracted separately from virions in spent cell culture media and served as templates for the construction of cDNA sequencing libraries using a NEBNext<sup>®</sup> Ultra<sup>™</sup> RNA library prep kit. The resulting cDNA libraries were sequenced on an Illumina MiSeq sequencer. Following removal of the host sequences (e.g., mosquitoes), de novo assemblies of the paired-end reads were performed. The BLASTX analyses of the assembled contigs identified the complete coding sequences (10 segments) for epizootic hemorrhagic disease virus (EHDV) strains, a novel strain of the Big Cypress orbivirus (BCPOV), a mobuck orbivirus (MBV), and three novel orbivirus species designated UF orbiviruses 1-3 (UFOV1-3). A Maximum Likelihood (ML) phylogenetic analysis based on the T2 protein amino acid sequence alignment of 33 orbiviruses supported UFOV1-3 as the closest relative to Guangxi orbivirus isolated from cattle (Bos taurus) in China in 2015. The isolation of BCPOV from a vertebrate host suggests it may represent a previously unknown mosquito-borne cervid pathogen. The isolation of MBV expands the known geographic range of this orbivirus into Florida. Further study is warranted to determine the vertebrate host range of these orbiviruses including their potential roles in disease among farmed and wild white-tailed deer populations.

#### A METABOLOMICS-BASED APPROACH TO ASSESSING HEALTH IN CAPTIVE BIGHORN SHEEP

Tara Stitzlein

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## Abstract

The health status of bighorn sheep (Ovis canadensis) remains incompletely understood despite extensive research on how disease, genetics, changes in nutrition and environmental factors have contributed to low rates of population recovery in these wild ungulates. Though these health parameters are being investigated and closely monitored in bighorn sheep populations by state agencies and other interest groups, the resulting information does not always translate in to clear answers to management questions. A relatively new approach to health uses Nuclear Magnetic Resonance (NMR) spectroscopy to detect and quantify bio-molecules called metabolites in blood serum. Metabolites result from the physiologic processes occurring in an organism, and can provide insights in to its health status due to their rapid and quantifiable response to physiologic changes, from dietary changes to disease processes. In this pilot study, we aim to develop an understanding of how metabolomics could be used in assessing bighorn sheep herd health by first applying this technology to a series of samples taken over a period of one year from a cohort of captive bighorn sheep lambs raised under controlled conditions and closely monitored for respiratory pathogens, changes in blood chemistry, nutrition state, parasite load and body condition. Resulting metabolic profiles will be compared over time concurrently with our defined health parameters, and investigated for changes related to immune response, disease state change, seasonal variation and environmental changes. Results from one year of analysis will be available in June 2019. A second cohort of lambs will be born in Spring 2019, with initial results from this series available by August 2019. These preliminary results will direct our future investigations in to how to use metabolomics to assess herd health and monitor disease processes.

# CANINE ADENOVIRUS TYPE-1 INFECTIONS IN CAPTIVE AND FREE-RANGING CALIFORNIA BLACK BEARS: CLINICAL OBSERVATIONS, PATHOLOGIC LESIONS, AND HISTORICAL EXPOSURE

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# Abstract

Between 2017 and 2018, four American black bear (Ursus americanus) cubs from the South Lake Tahoe area died from infectious hepatitis and encephalitis resulting from canine adenovirus type 1 (CAV-1) infection. The initial diagnoses were confirmed on two orphaned cubs from a local wildlife rehabilitation facility in August and September of 2017. Endothelial and hepatocellular inclusion bodies suggestive of adenovirus were noted in brain and liver, respectively. CAV-1 infection was confirmed by immunohistochemistry, polymerase chain reaction (PCR) assays, virus isolation, and electron microscopy on affected tissues and cell cultures. Serologic testing on four cubs that shared the same enclosure at the rehabilitation facility indicated that all the cubs had been exposed to CAV-1 and seroconverted. Shedding of virus through the urinary tract was demonstrated by detecting adenovirus in the urine of one cub by polymerase chain reaction (PCR) assay. Following these initial diagnoses, we screened all recent black bear diagnostic cases that had hepatitis for CAV-1 by immunohistochemistry. Hepatitis due to CAV-1 was confirmed in a free-ranging black bear cub that died in the South Lake Tahoe in June 2017, preceding the initial two rehabilitation cases by several months. A second free-ranging cub from the South Lake Tahoe area died from CAV-1 infection in October 2018. Other likely causes of encephalitis and hepatitis were ruled out via histopathology and ancillary diagnostics. Serosurveillance conducted on a sub-set of archived black bear serum samples from across California indicated persistent but low levels of exposure throughout the state's free ranging black bear population. Here we describe clinical observations, post-mortem lesions, and ancillary diagnostics confirming CAV-1 infections as the cause of mortalities in free-ranging and captive (wildlife rehabilitation) black bear cubs in California.

#### CHRONIC WASTING DISEASE IN MISSOURI: WHERE ARE WE NINE YEARS LATER?

Jasmine Batten, Sherri Russell

Missouri Department of Conservation

# Abstract

Chronic wasting disease (CWD) was first detected in Missouri in big game hunting preserves in 2010 and 2011 and in 5 free-ranging deer within 2-miles of one of the facilities in 2012. The Missouri Department of Conservation (MDC) implemented an aggressive response centered on detecting CWD early, monitoring changes, and minimizing the impacts of the disease over time. To meet these goals, MDC has passed regulations, increased surveillance dramatically (over 800% since 2014), and implemented post-season targeted culling (sharp-shooting) where CWD is found. Despite these efforts, CWD has been found in 15 additional counties with a uniquely discontinuous pattern. The continued spread of CWD into new areas of the state may be perpetuating the idea that CWD spread in Missouri is out of control and there is little we can do to slow it down. The reality is, however, CWD prevalence is low where it has been detected, even in northeast Missouri where the disease was first detected. While MDC's commitment to minimizing the impacts of CWD remains firm, meeting the goals of early detection and active management is becoming increasingly challenging. The greatest immediate barriers to successful CWD management in Missouri include addressing the movement and improper disposal of carcasses into and within Missouri; continuing to reduce the risks of disease spread associated with captive wildlife; maintaining public support for CWD efforts (especially post-season targeted culling); developing more effective communication tools to better counter misinformation and false narratives; and preventing agency "CWD fatigue" to keep staff on board and engaged. Fortunately, the majority of the deer in Missouri remain unaffected today, and the majority of hunters in Missouri are concerned about CWD. Therefore, opportunity remains to positively influence the course of CWD in Missouri overtime.

# TRICHOPHYTON EQUINUM ASSOCIATED WITH SEVERE DERMATOPHYTOSIS IN AMERICAN BLACK BEARS FROM CALIFORNIA

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## Abstract

From 2014 to 2018, we evaluated six juvenile American black bears (Ursus americanus) with severe dermatopathy, emaciation, and weakness. All bears had severe, often generalized exfoliative and crusting dermatitis and alopecia. Two cubs died while in care and four were euthanized due to poor prognosis. Histology of the skin revealed acanthosis with hyperkeratosis and pleocellular dermatitis with folliculitis and furunculosis. Hair and follicles from all regions examined, were heavily colonized by fungal spores and hyphae, which were also present in superficial serocellular crusts. Fungal cultures performed on multiple skin sites (neck, flank, dorsum, limbs) from each bear yielded isolates of Trichophyton sp. most consistent with T. equinum. Mites consistent with Uriscoptes sp. and Demodex sp. were detected in three of the bears via skin scrapings or histology. Trichophyton sp. isolates were submitted to the University of Texas Fungus Testing Laboratory for further characterization. Phenotypic assessments and DNA sequencing of the ITS1, ITS2, and 28S D1/D2 regions of ribosomal DNA were used for species identification. Additionally, random amplified polymorphic DNA (RAPD) analysis determined that isolates from all six bears were distinct from the quality control T. equinum strain but indistinguishable from each other. Anecdotal evidence suggests the occurrence of severe skin disease and hair loss in cub and yearling black bears in California is increasing. This work demonstrates that a clonal T. equinum strain produces severe disease in these juvenile animals. The significance of dermatophytosis due to T. equinum in California black bear populations and the role it may play as a sole causal agent or as a co-pathogen in other diseases warrants further investigation.

# DETECTION OF ANTIMICROBIAL RESISTANT BACTERIA IN WILD BIRDS ASSOCIATED TO AN URBAN COLONY OF SEA LIONS IN CHILE

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#### Abstract

Bacterial resistance to antimicrobials has a significant role in human and animal health, as it leads to therapeutic failure and to the development of multiresistant microorganisms with ability to transmit this resistance to potentially harmful bacteria. The aims of the present work were to isolate, identify and characterize extended spectrum beta-lactamases producing enterobacteria from feces of wild birds: turkey vulture (Cathartes aura), black vulture (Coragyps atratus), brown-hooded gull (Chroicocephalus maculipennis) and cormorant (Phalacrocorax brasilianus), associated to a colony of urban sea lions in the city of Valdivia, Chile. To achieve these goals, 101 samples (25 cormorants, 25 brown-hooded gulls and 51 turkey and black vultures) were taken from the area where these birds and the sea lions interact. Samples were obtained and then processed at the Animal Pathology Institute at the Universidad Austral de Chile. The bacteria isolation was made in an ESBL-specific media to later identify bacterial colonies and to perform DNA extraction, to characterize the main genes that encode for extended spectrum beta-lactamases (TEM, OXA, SHV and CTX-M) through PCR. Approximately, 48% of all the samples showed growth of ESBL-producing enterobacteria colonies, confirmed to be Escherichia coli. Results indicated that the genes encoding for ESBL enzymes that occurred most frequently were CTX-M and TEM. Vultures presented 63% (32/51) samples with ESBL-producing colonies. Furthermore, the resistance pattern observed in the vultures corresponds to results obtained a previous study from the sea lions in the area, and could be explained due to their coprophagic behaviour. The present study confirms the presence of ESBL bacteria in most of the fecal samples collected. Also, enzymes that give resistance to these bacteria were identified. Furthermore, a potential source and flux from resistant bacteria in the studied environment was also recognized, increasing the risk of acquiring these antibiotic resistant bacteria by the human population.

# WHITE STORKS (CICONIA CICONIA), LANDFILLS AND EXTENDED-SPECTRUM BETA-LACTAMASES-PRODUCING ESCHERICHIA COLI

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# Abstract

White storks (Ciconia ciconia) are large birds that connect Europe, Africa and parts of Asia through their migratory flyways. Recent studies on the movement ecology of storks report ample effects of landfills on stork ecology, life histories and behavior. The combination of epidemiology and movement ecology tools offers unique possibilities to understand how zoonotic diseases may spread, the location of potential sources and risk areas. Between 2011 and 2014, we sampled white stork nestlings from colonies along a gradient of exposure to landfill foraged food, with the objective of assessing the potential of the species to act as reservoir and vehicle of cephalosporin resistant (CR) Escherichia coli. E. coli isolates were phenotypically characterized, presence of CR genes was confirmed and plasmids were classified. Risk factors for acquiring these genes were assessed. Overall, 8.8% (41 out of 467) storks carried CR E. coli in their cloaca and five were identified from recently deposited droppings; therefore 46 isolates were further characterized. Of these, 21 contained blaCTX-M-1, 10 blaCMY-2, 6 blaCTX-M-14, 3 blaSHV-12, 3 blaCTX-M-15, 2 blaCTX-M-1 together with blaCMY-2, and 1 blaCTX-M-1 together with blaSHV-12. All were multi-resistant, and three harboured the plasmid-mediated colistin resistance mcr-1 gene. CR genes were associated with the presence of Incl1, IncFIB and IncN replicon families. Pulsed-field gel electrophoresis demonstrated a high degree of polymorphism, but showed also identical profiles from isolates obtained from different locations. Carriage of CR E. coli was strongly associated to use of landfills. This study demonstrates that the proximity of white storks to human activities with high antimicrobial pressure contributes to the acquisition and dissemination of CR E. coli.

# COMPLETE GENOME SEQUENCING OF CETACEANPOX VIRUS FROM A MANAGED INDO-PACIFIC BOTTLE-NOSE DOLPHIN (TURSIOPS ADUNCUS)

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#### Abstract

Cetacean poxviruses (CePVs) are associated with cutaneous disease in wild and managed cetaceans often referred to as "tattoo" lesions. Only partial genomic data are available for CePVs, and thus, they remain unclassified members of the subfamily Chordopoxvirinae, family Poxviridae. Herein, we describe the complete CePV genome sequenced from a managed Indo-Pacific bottlenose dolphin (Tursiops aduncus) displaying tattoo-like lesions. A deep swab from the lesion was collected and frozen at-80oC. DNA was extracted from the sample using a QIAamp Viral DNA Mini Kit, a DNA library was generated, and sequenced on a MiSeq sequencer. The complete T. aduncus CePV genome was assembled and annotated in CLC Genomics Workbench. The CePV genome was determined to be 121,769 bp, and predicted to encode 120 proteins. All 49 poxvirus and 31 chordopoxvirus conserved core genes were identified, along with 10 genes unique to the CePV and 4 genes that appear to function as immune-evasion genes. The CePV genome represents the smallest poxvirus genome sequenced to date with the next two smallest genomes determined from the other marine mammal poxviruses, the sea otterpox virus (SOP-ELK; 127,879 bp) and seal parapoxvirus (127,941 bp). Genetic and Maximum Likelihood phylogenetic analyses based on the concatenated amino acid (aa) sequence alignment of 7 conserved core poxvirus proteins were performed. The aa sequence identity of CePV to other known chordopoxviruses ranged from 34.8-71.1%, with highest identity to members of the genera Cervidpoxvirus. The phylogenetic analysis supported the CePV as a unique branch among chordopoxviruses. Based on these results, we propose that CePV represents a new poxvirus species, and likely belongs in a new genus, pending consideration by the International Committee on Taxonomy of Viruses. The genomic characterization of the CePV provides an important update to their taxonomy, and molecular information for development of more specific and sensitive diagnostic techniques.

#### PATTERNS OF PESTIVIRUS IN CARIBOU AND MUSKOXEN: DOES GEOGRAPHY AND ECOTYPE MATTER?

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## Abstract

Ruminant pestiviruses, Border Disease Virus (BDV) and Bovine Viral Diarrea Virus (BVDV), are production limiting pathogens for the domestic livestock worldwide. The highly mutagenic nature of these RNA viruses causes a wide variety of infection outcomes, including subclinical transient viremia, abortion, respiratory problems or lymphoid depletion that predisposes to secondary fatal infections. The persistence of ruminant pestiviruses within wild free-ranging populations is suspected for BVDV in white tailed deer (Odocoileus virginianus), and for BDV in Pyrenean chamois (Rupicapra pyrenaica), with the severe chamois population declines caused by BDV outbreaks being noteworthy. Little is known about pestivirus in wild caribou (Rangifer tarandus) or muskoxen (Ovibos moschatus), yet serosurveys in North America indicate that pestiviruses are circulating in these species. The absence of livestock across muskox and caribou range suggests that viral strains may be self-maintained among these species. In general, seroprevalence in caribou is much higher than in muskoxen. Among caribou ecotypes, spatial ecology and behavior appear to influence the dynamics and persistence of pestivirus. Migratory tundra caribou (R. t. groenlandicus and R. t. granti), that move and calve en masse, tend to have higher seroprevalences (34%-78%) compared to caribou ecotypes that calve individually and/or form smaller aggregations or social groups, for example Dolphin and Union caribou (R. t. groenlandicus x pearyi) (15%-23%) or non-migratory woodland caribou herds (R. t. caribou) (0%-1%). The identity of the pestivirus(es) circulating among caribou and muskoxen, the impacts on health at individual and population level, and the epidemiology of these viruses in a host-community sense, including all susceptible and sympatric species, remain unknown. Herein we will review published and unpublished data of pestivirus in caribou and muskoxen across their range and examine its epidemiology in relation to ecological and behavioral traits.

# LEPTOSPIROSIS AND DETECTIONS OF LISTERIA MONOCYTOGENES, TOXOPLASMA GONDII, SARCOCYSTIS CANIS IN A KODIAK BROWN BEAR

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## Abstract

Determination of Cause of death (COD) in brown bears in Alaska has been difficult due to the remoteness of their habitats, low human population levels and thus the ability to access and detect carcasses quickly. A fresh dead male, 8-month old Kodiak brown bear (Ursus arctos) was found on October 21, 2018. This animal was dehydrated, in poor body condition and had multifocal random hepatitis and nephritis and probable pneumonia on gross examination. By aerobic cultures at Athens Veterinary Diagnostic Laboratory (AVDL), liver, kidney and brain were negative and the lung positive for Aeromonas salmonicida, Listeria monocytogenes, and Serratia fonticola. On histopathology, major findings included severe pulmonary edema and atelectasis, mononuclear hepatitis and hepatocellular necrosis and severe lymphoplasmacytic tubulointerstitial nephritis, and large numbers of protozoal cysts in the muscle and enterocytes. Both kidney and liver were positive for Leptospira sp. by polymerase chain reaction (PCR) at AVDL. Sequencing was complicated by bacterial contamination and yielded possible species of L. interrogans, santarosai, borgpetesenii, noguchi, mayottensis, kirscheneri, weilii. Toxoplasma gondii was also detected in the brain and liver and Sarcocystis canis, was identified in muscle, tongue and liver by PCR-DNA sequencing the ITS1 region to identify the parasite species present at the National Institute of Health (NIH). The strong signal and liver infection with T. gondii supports an acute infection, but the lack of histopathology does not support a role in the mortality. Ultimate COD was determined to be aspiration pneumonia and the proximate COD clinical leptospirosis possibly complicated by acute Toxoplasma infection. This represents the first reported case of clinical leptospirosis in a bear, and characterization of T. gondii and isolation of Listeria monocytogenes in an Alaskan brown bear, all significant zoonotic diseases as well as S. canis, a parasite known to cause a necrotizing hepatitis in bears.

#### ANTLER DEFORMITIES IN MULE DEER BUCKS IN THE PAUNSAUGUNT AREA OF UTAH, USA

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## Abstract

Antler deformities in mule deer (Odocoileus hemionus) may be caused by multiple factors, including injury to the pedicles or reproductive organs, nutritional deficiencies, hormonal alterations, or infectious disease. The Paunaugunt area of southern Utah has a high concentration of mule deer bucks with antler deformities ("cactus bucks"). We investigated possible causes by conducting full post mortem examinations on 10 hunter-harvested and 10 live-caught mule deer bucks with antler deformities in November of 2016-18. As a comparison, 6 hunter-harvested and 43 live-caught mule deer bucks without antler deformities were sampled from the same area and time periods. All bucks were screened by serology for exposure to epizootic hemorrhagic disease virus, bluetongue virus, Brucella abortusand Brucella ovis, caprine arthritis and encephalitis virus, and bovine viral diarrhea virus. Further, serum testosterone and trace minerals concentrations were measured, and whole blood counts evaluated. PCR for EHDV-1, 2, and 6 was conducted on 2 testicular tissues and 10 whole blood samples. End-stage fibrosing orchitis was observed in all bucks with antler deformities, and one testicular sample tested positive for EHDV-2 on PCR. All bucks with antler deformities had low to non-detectable levels of testosterone. Seropositivity for EHDV-2 was observed in 7/7 live-captured bucks and 8/10 hunter-harvested bucks with antler deformities, and 15/24 and 2/6 normal live-caught and hunter-harvested bucks. A total of 25/30 normal and 16/17 abnormal bucks tested seropositive for BVDV-2. [Results from 2018 (3 live bucks with antler deformities and 18 live normal bucks) were still pending at time of abstract submission]. Copper deficiencies were detected in 15/16 hunter harvested animals. Our results support the hypothesis put forth by other researchers, that infection with EHDV-2 may play a role in the development of antler deformities. However, it is still unclear why some infected bucks develop antler deformities while other do not.

# GENERAL POSTERS END

# EMERGENCY CAPTURE OF THE CRITICALLY ENDANGERED VAQUITA (PHOCOENA SINUS)

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# Abstract

In 2017 an emergency field effort was undertaken in an attempt to prevent the extinction of the world's most endangered marine mammal, the vaquita (Phocoena sinus). After a long decline from entanglement in legal gillnet fisheries, the vaquita population fell from more than 200 in 2008 to fewer than 30 in 2016, due to entanglement in an illegal gillnet fishery that supplies swim bladders of the endangered totoaba (Totoaba macdonaldi) to Chinese black markets. An emergency ban of gillnets and increased enforcement failed to slow the decline, triggering the emergency effort to catch vaquitas and place them under protection in captivity. Two animals were captured using light gill nets; a juvenile was released after four hours because it appeared stressed (plasma cortisol was 23.4 µg/dL) and an adult female died of capture myopathy (cortisol 150 ug/dL, aldosterone 237 pg/ mL, epinephrine 31,615 pg/mL). The program was suspended because of the risk of additional mortalities to the population. The lack of success in capturing vaquitas for temporary protection emphasizes the need to improve our understanding of the effects of chase, capture, handling and enclosure on cetaceans, and to consider intervention before populations reach critically low levels, when there is sufficient time to use phased, precautionary approaches. Furthermore, conservation approaches focused on single species must be integrated into broader efforts to conserve ecosystems and involve the human communities that depend on them.

#### **RETROSPECTIVE STUDY OF CHANNEL ISLAND FOX MORTALITY: 2010-2018**

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## Abstract

Case records from the California Animal Health and Food Safety Laboratory (CAHFS) were reviewed for all Channel Island foxes submitted to CAHFS for postmortem examinations from 2010 through 2018. During this period, 384 foxes were examined from six islands representing six subspecies. Accessions were assessed for cause of death where possible and contributing and/or concurrent diseases. Of 384 foxes examined, 136 were submitted from San Clemente Island, 130 from Santa Catalina Island, 49 from San Miguel Island, 38 from San Nicolas Island, 17 from Santa Rosa Island and 14 from Santa Cruz Island. The most common cause of death was vehicle trauma (174/384). Disease trends of the foxes varied between islands. In San Clemente Island foxes (U. littoralis clementae), cholecalciferol toxicosis (15/136) and moderate to severe pneumoconiosis (54/136) were unique to this island. In San Miguel Island foxes (U. littoralis littoralis), internal parasitism was the most significant disease and highly associated with emaciation. Enteric parasites in this subspecies included a newly described Acanthocephalan sp. (26/49) associated with enteritis and occasionally peritonitis. Intramural colonic Spirocerca sp. (37/49) caused colitis and occasionally peritonitis and peritoneal hemorrhage. Pulmonary and/or vascular parasites were detected in 24 of the 49 San Miguel Island foxes examined. Colitis due to Spirocerca sp. was also prevalent in Santa Rosa Island foxes (U. littoralis santarosae), Santa Cruz Island foxes (U. littoralis santacruzae), and San Nicolas Island foxes (U. littoralis dickeyi) with infections most severe in the latter. Localized or systemic amyloidosis was present in foxes on San Clemente Island (12/136), Santa Catalina Island (8/130), and Santa Cruz Island (1/14). Ceruminous adenocarcinomas were seen only in foxes on Catalina Island (11/136).

This study demonstrates the wide variation in disease exposures and outcomes between U. littoralis subspecies which may be related to genetic susceptibility, environmental factors or concurrent infections.

# EVIDENCE FOR SEA TURTLE COLD-STUNNING ALONG THE PACIFIC COAST OF THE UNITED STATES AND CANADA

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# Abstract

As ectothermic marine reptiles, sea turtles are sentinels for global climate change and their stranding patterns reflect real-time shifts in oceanographic conditions. Sea turtles may experience cold-stunning when ocean temperatures drop below a threshold of 10-12 °C, causing hypothermia and reduced responsiveness. This phenomenon has been well described in the northwest Atlantic Ocean, where annual mass stranding events occur seasonally during winter months following a rapid reduction in ocean temperature. However, a case definition for this condition that considers regional environmental conditions and species differences in thermal tolerance has not yet been developed for the Pacific coast. The goals of this study were to analyze historical data on sea turtle stranding patterns relative to environmental parameters that may contribute to cold-stunning and characterize the health status of affected individuals. All available data on live sea turtle strandings (n=95) from 2009 to 2018 collected by the NOAA Fisheries West Coast Region and the Department of Fisheries and Oceans Canada were evaluated for inclusion. Species included the east Pacific green turtle (Chelonia mydas), north Pacific loggerhead (Caretta caretta), and Pacific olive ridley (Lepidochelys olivacea). Cases were excluded if turtles were not recovered for examination, or if they stranded as a direct result of human interaction (ie. vessel strike, fisheries interaction, marine debris ingestion, or power plant entrainment). For all remaining cases (n=51), associated environmental data and medical records were compiled for analysis. Minimum daily sea surface temperature at the closest NOAA buoy was recorded on the date of stranding and weekly for 4 weeks prior to stranding. A summary of significant clinical and postmortem findings is presented to facilitate comparison with published literature. These data provide compelling evidence for cold-stunning and a valuable baseline from which to monitor the impacts of changing ocean conditions on these federally protected species.

# PATHOLOGY AND EPIDEMIOLOGY OF NASOPULMONARY ACARIASIS (HALARACHNE SP.) IN SOUTHERN SEA OTTERS (ENHYDRA LUTRIS NEREIS)

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## Abstract

Halarachne sp. nasal mites infest harbor seals (Phoca vitulina) and southern sea otters (Enhydra lutris nereis) in California, but little is known about the pathophysiology of these infestations, or risk factors for exposure. To investigate these questions, a retrospective case-control study was performed using necropsy data from 70 mite-infested sea otters, and 144 non-infested controls. Case records for sea otters examined by pathologists from February 1999 through May 2015 were examined to assess risk factors for infestation, and lesions associated with nasopulmonary acariasis. Animals with a history of captive care within 10 days of death or carcass recovery were 3.2 times more likely to be infested with nasopulmonary mites than those with no history of recent rehabilitation. Sea otters stranding within 1 km of Elkhorn Slough in Monterey Bay were 4.9 times more likely to be infested with nasal mites than other areas; this site is characterized by high sea otter contact with sympatric harbor seals (a common host for Halarachne sp.), and a comparatively large population of rehabilitated and released sea otters. Aged adult otters were 9.4 times more likely to be infested than younger animals, and sea otters with nasopulmonary acariasis were 14.2 times more likely to have upper respiratory inflammation than un-infested animals. Additional findings in otters with nasopulmonary acariasis included lower respiratory tract bacterial infections, presence of medium-sized and/or fresh nose wounds at necropsy (indicators of recent face-to-face interaction between otters during copulation or fighting), and turbinate bone erosion. Our findings, although preliminary, suggest that captive rehabilitation and close contact with harbor seals could facilitate nasopulmonary mite transmission to sea otters. We also identified a high-risk zone for nasopulmonary acariasis in sea otters. We also provide preliminary data to suggest that nasopulmonary mite infestations can cause significant respiratory pathology in sea otters.

#### **INVESTIGATING SOUTHERN SEA OTTER (ENHYDRA LUTRIS NEREIS) MORTALITY PATTERNS (1998-2012)**

Melissa Miller<sup>1</sup>, Megan E. Moriarty<sup>2,3</sup>, Erin M. Dodd<sup>1</sup>, Tristan Burgess<sup>4</sup>, M. Tim Tinker<sup>5</sup>, Francesca Batac<sup>1</sup>, Laird A. Henkel<sup>1</sup>, Colleen Young<sup>1</sup>, Mike Harris<sup>1</sup>, Frances Gulland<sup>3</sup>, Pádraig Duignan<sup>6</sup>, Raphael M. Kudela<sup>7</sup>, Christine K. Johnson<sup>3,4</sup>

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## Abstract

Understanding causes of mortality in wildlife is critical for effective population management and veterinary care. We compiled data from 560 southern sea otter (SSO; Enhydra lutris nereis) necropsies spanning 15 years (1998-2012). Our sample population included minimally decomposed, subadult (1-4 years), adult (4-10 years), and aged adult (>10 years) SSO, including both random-source stranded, and radio-tagged study animals. Primary and contributing cause(s) of death were defined by gross lesions, histopathology and diagnostic tests. We distinguished between primary or contributing cause(s) of death, and sequelae such as secondary bacterial infection. In addition to updating collective knowledge regarding classical causes of SSO death, such as bites by white sharks (Carcharodon carcharias), protozoal infection and acanthocephalan peritonitis, we also evaluated less well-characterized processes such as domoic acid (DA) intoxication, cardiomyopathy and end-lactation syndrome (ELS) that could be impacting SSO population recovery. Spatial scan statistics and multivariate models were used to identify high or low-risk locations for SSO stranding with common diseases, and associations between common disease processes. Potential risk factors that were assessed included sex, age, and stranding season. Shark bite was the most common primary cause of death, followed by acanthocephalan peritonitis, probable DA intoxication, protozoal infection, cardiomyopathy, ELS, and primary bacterial infection. When the primary and top three contributing cause(s) of mortality were pooled, the most common cause(s) of death were gastrointestinal erosions/melena, cardiomyopathy, probable or possible DA intoxication, shark bite, emaciation, acanthocephalan peritonitis, protozoal infection, ELS, and primary bacterial infection. Significant spatial, temporal, or space-time clustering was noted for several common causes of death. Substantial forward progress was made to develop a provisional case definition for DA intoxication as a cause of SSO death, clarify associated cardiac lesions, and provide a temporal estimate of disease chronicity.

# WHITE ABALONE (HALIOTIS SORENSENI) RESILIENCE IN THE FACE OF EXTINCTION: MITIGATING DISEASE IMPACTS ON ENDANGERED ABALONE IN A CAPTIVE BREEDING PROGRAM

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## Abstract

White Abalone (Haliotis sorenseni) is an ecologically- and culturally-important marine species on the brink of extinction. The abundance of the species, which is native to the waters off of Southern California and Baja California, significantly declined as a result of overfishing, with surveys of established populations showing a 99% decrease since the 1970s. Even with the closure of the commercial fishery in 1997, populations continued to decline and white abalone were federally listed as endangered in 2001, becoming the first marine invertebrate to receive that designation. Densities of the remaining population, estimated to be less than 3,000 individuals, appear to be too low to facilitate the broadcast spawning necessary to perpetuate the species without human intervention. In 2008, the White Abalone Recovery Plan was released by NOAA to restore the species by establishing self-sustaining populations. A captive breeding program—housed at several institutions, including UC Davis' Bodega Marine Laboratory—has been successful in producing a new generation destined to be outplanted in the wild. However, Withering Syndrome, a chronic degenerative disease caused by the rickettsiales prokaryote Candidatus Xenohaliotis californiensis (CaXc), presents a grave threat to laboratory-raised animals planted in the wild. To develop a greater understanding of when white abalone may be the most vulnerable to infection with CaXc, we've undertaken an experiment using one-, two-, and three-year-old abalone to determine whether age at outplanting may influence susceptibility to CaXc infection and disease expression. To expand the culturing capacity of the program and investigate the effect of exposure to non-sterilized seawater at an earlier age, we are also conducting an experiment to explore the effects on culturing white abalone under typical abalone farm conditions. Both these experiments will inform forthcoming outplanting efforts and aid in species recovery in the wild.

# WHERE TO LOOK FOR FLAVIVIRUSES IN WILDLIFE? MACHINE LEARNING TO INFORM LARGE SCALE SURVEILLANCE OF WILDLIFE FOR EMERGING FLAVIVIRUSES

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# Abstract

Flaviviruses remain an important global health threat and have clear implications for wildlife conservation, especially for endangered wildlife populations susceptible to sylvatic transmission. Sylvatic cycles of flaviviruses play a significant role in maintaining transmission in regions vulnerable to outbreaks and hence it is critical to identify potentially as yet unobserved sylvatic reservoirs to inform long-term disease control, especially for emerging flaviviruses such as Zika virus (ZIKV). We address the challenge of informing large-scale surveillance activities by investigating current knowledge of flaviviral hosts and determining which ecological, physiological and climatic conditions of known hosts help identify potentially unobserved hosts of flaviviruses. We detected 140 mammals and 277 avian species as recognized hosts for flaviviruses. Flaviviruses showed a wide range of propensity for host plasticity with a mean of 18.9 host species (range 1-194) per virus. We developed machine-learning models that use host-traits applicable to all mammalian and avian species and account for vector distribution. Using this modeling framework, we predicted potential as yet-unobserved hosts for seven flaviviruses and viral groups (flaviviruses known to infect similar hosts) with high accuracy (AUC range 0.66-0.99). We were able to identify regions of the world with high richness of potential hosts that could facilitate sylvatic transmission for viruses emerging or endemically circulating in these regions. For example, the model for ZIKV and yellow fever virus (YFV) predicted thirteen potential primate hosts that have yet to be detected positive, and we identified high levels of host species richness in Southeast Asia. Additionally, high richness of potential bird host species for West Nile virus (WNV) was highlighted in Europe, which could enhance WNV transmission and maintenance in the wake of current outbreaks in this region. Our findings narrow down species targets and regions of the world for surveillance of flaviviruses to better understand flaviviral ecology in wildlife.

#### MODELING LAND-SEA TRANSMISSION OF TOXOPLASMA GONDII

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# Abstract

The threatened Southern sea otter (Enhyra lutris nereis) has proven an excellent study species for understanding pathogen pollution in its nearshore habitat. Behavioral, dietary and environmental risk factors for Toxoplasma gondii infection in sea otters have been described, but some of these variables are likely proxies – variables with no causal role, but which co-occur with causal risk factors. In vitro evidence, combined with epidemiological analysis of risk factors, suggests a transmission pathway involving freshwater and marine transport of infectious oocysts, aggregation mediated by marine exopolymers, deposition on the surface of kelp fronds, and oocyst concentration by kelp-grazing marine snails (Tegula spp.). We aim to test existing theory using mechanistic modeling and real-world data. To this end, we constructed a mixed mechanistic-probabilistic model of the transmission of T. gondii from felid definitive host shedding through to sea otter infection, including linked models of oocyst loading/mobilization, oocyst transport and infection risk. We fit this model to a 15-year dataset of individual sea otter home ranges, diets and T. gondii serological testing data spanning almost all of the documented range of the Southern sea otter. By using this model to estimate the effect of changes in risk factors on whole population prevalence, we found that sea otter T. gondii prevalence was sensitive to changes in oocyst loading and marine habitat (such as hard vs. soft substrate and presence of kelp beds) but that terrestrial watershed variables were not informative, independent of their effects on oocyst loading. Associations of high T. gondii prevalence with freshwater outflow, high human population density and developed land use appear to be proxies for increased oocyst loading into the marine environment. Risk was highest in areas of rocky and kelp-dominated habitats near the mouths of rivers, though high-risk at the mouths of small rivers were smaller in extent.

# SATELLITE TRACKING OF GULLS AND WHOLE GENOME SEQUENCING OF E. COLI PROVIDES INFERENCE ON THE EPIDEMIOLOGY OF ANTIMICROBIAL RESISTANT BACTERIA IN WILD BIRDS IN ALASKA

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# Abstract

Antimicrobial resistant (AMR) bacteria are a global threat, with an increasing number of infections no longer responding to once-standard treatments. Although antimicrobial resistance is naturally occurring, antibiotics and other anthropogenic exposures have selected for resistance in commensal and pathogenic bacteria in diverse hosts and environments. The source of AMR bacterial infections is often unknown, as is the environmental distribution and prevalence of resistance genes pertinent to human and animal health. Previous surveys suggest that some types of birds, such as landfill foraging gulls (Larus spp.) and scavenging birds of prey, may be useful indicators of antimicrobial resistance in the environment. However, the pathways governing the acquisition and dispersal of such bacteria by birds are not well-described. We investigated the role of wild birds in the epidemiology of AMR bacteria in Alaska. Specifically, we aimed to identify genetic determinants conferring resistance, explore potential transmission pathways of AMR bacteria and genes in relation to local gull movements, and compare genetic diversity to isolates reported in other taxa. We equipped 17 gulls with satellite transmitters and performed whole genome sequencing of >200 AMR E. coli isolates recovered from gull and bald eagle (Haliaeetus leucopehalus) feces collected at sites throughout Alaska. We found extensive genetic diversity of E. coli isolates and antimicrobial resistance genes and also evidence for strain sharing among bald eagles and gulls through time and space. Satellite tracking of gulls revealed that the genetic population structure of AMR E. coli originating from samples collected on the Kenai Peninsula generally mirrored gull movements. Concerningly, multidrug-resistant isolates were found, including carbapenemase-producing E. coli. Our findings support complex epidemiological interactions, including bacterial strain sharing between birds, horizontal gene transfer among E. coli harbored by birds, and maintenance and dispersal of AMR E. coli of public health importance by wild bird populations in Alaska.

## INFLUENZA VIRUS TRANSMISSION IN A MULTISPECIES BARNYARD MODEL

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## Abstract

Influenza A viruses are a diverse group of pathogens that infect a wide variety of animals and have been responsible for millions of human and avian deaths throughout history. The viruses are well adapted to avian hosts and can spread quickly from one host to the next. Our hypothesis is that select species of birds are more competent to shed and transmit the virus than others and the environmental conditions that favor transmission are often present in barnyards where avian species intermix. In these experiments, we illustrate the transmission potential between Coturnix quail, domestic ducks, chickens, and house sparrows, all of which were co-housed and shared common food and water sources. The wild birds (either quail or sparrows) were infected with one of several H7 influenza viruses and introduced into the barnyard with the other animals. Oral and/or cloacal swabs were collected from all animals in the room for up to 12 days and serum was collected terminally to assess seroconversion. Additionally, water samples were tested for the presence of live virus daily for up to 12 days. Initial results demonstrate that Coturnix quail, which are found in the wild but are often kept as poultry, shed virus orally within 24 hours of exposure and are a source of infection for other birds. Sparrows also shed the virus orally, but at lower concentrations and for a shorter duration of time, making them less apt to spread the virus. These results, combined with previous work demonstrating transmission from chickens to quail in an artificial market setting, suggest that quail are a species of interest for the transmission of avian influenza viruses. As such, minimizing contact between domestic birds and quail and maintaining clean facilities with separate food and water sources between species could reduce the possibility of interspecies transmission.

#### **RETHINKING THE WILDLIFE HEALTH RESEARCH ENTERPRISE WITH A FOCUS ON SOLUTIONS**

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#### Abstract

Scientific research falls broadly into two categories: curiosity-driven or problem-focused. The majority of funded and published wildlife health research is problem-focused, yet relatively little progress has been made in the deployment of effective solutions for those problems. The field of wildlife health is not alone in the challenge of translating basic scientific research into real-world solutions, and a growing chasm between research activity and improvements in human health has driven the development of translational research over the past 15 years. Translational frameworks are essentially a fundamental restructuring of the research enterprise, demanding the formation of highly multidisciplinary research groups, the creation of interdisciplinary translational expertise, and a shift in the emphasis of funding and publication towards later phases of research focused on the efficacy and real world effectiveness of solutions.

Translational theory in human health is drawn upon to create a translational framework for wildlife health research, synthesising commonalities and addressing features unique to wildlife health. This framework introduces four broad translational phases situated on a bidirectional continuum of research activity stretching from problem identification to the deployment of effective solutions. These phases are: problem definition (T0); and the development of potential solutions (T1), efficacious solutions (T2), and effective solutions (T3). A systematic review of recent literature on wildlife health demonstrates a dramatic bias towards early T1 phase research.

I argue that the roadblocks to solving problems of wildlife health lie not only in the development of potential biomedical solutions to wildlife disease (phase T1), but also in the earliest (problem definition) and the latter (developing efficacious and effective solutions) translational phases, and that solution-finding in wildlife health has neglected the challenges and opportunities lying in the field of human sociology across all translational phases. I propose a fundamental restructuring of problem-focused wildlife health research to overcome these roadblocks.

#### MODELING BACILLUS ANTHRACIS IN TEXAS AND ESTIMATING DEER RISK IN THE ENZOOTIC ZONE

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## Abstract

Ecological niche modeling approaches have been applied to model the potential geographic distribution of Bacillus anthracis from local to national scales on many landscapes, including the contiguous US and local outbreak zones within the US. To date, national models in the US have modeled the pathogen across genetic lineages and singly the A1.a lineage. Here, we revise risk estimates with a mosaic approach, first modeling local patterns of specific lineages in Texas, Montana, and the Midwestern states and piece those predictions together into a single risk map for B. anthracispersistence. Texas has a high diversity of lineages, with frequent outbreaks driven by the A4 (Vollum lineage) and Ames near neighbors (A3/Ames-like lineage) with some A1.a cases. In the northern states, such as the Dakotas and Montana, the A1.a lineage is dominant. Here we model and compare each lineage across its own geography and mosaic a single map to improve risk predictions. We then evaluate the estimated deer population for Texas and estimate the population within the newly redefined risk zone. This will allow managers and wildlife managers to better plan surveillance and control in the newly redefined enzootic zone.
### BORRELIA BURGDORFERI IN UPLAND GAME BIRDS FROM PENNSYLVANIA, USA

Christopher Cleveland<sup>1</sup>, Liandrie Swanepoel<sup>2</sup>, Justin Brown<sup>3</sup>, Mary Jo Casalena<sup>4</sup>, Lisa Williams<sup>4</sup>, Michael Yabsley<sup>2</sup>

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# Abstract

The Borrelia genus can be separated into two major clades, the Lyme borreliosis group, which includes the causative agents of Lyme disease, and the relapsing fever borreliosis group. In North America, several Lyme disease and relapsing fever Borrelia spp. are important pathogens of veterinary and public health concern. Lyme disease is a significant interest in the northeastern United States because incidence continues to increase in both dogs and humans. Many avian species may play an important role in the ecology of Lyme disease because they can serve as hosts for tick vectors and competent hosts for Borrelia species. The goal of this study was to investigate the prevalence of Borrelia species in four species of upland game birds: wild turkey (Meleagris gallopavo), ruffed grouse (Bonasa umbellus), ring-necked pheasants (Phasianus colchicus), and American woodcock (Scolopax minor) from Pennsylvania, USA. A total of 205 tissue samples (bone marrow and/or spleen samples) from 169 individuals were collected from free-living, hunter-killed individuals. Borrelia was detected using a flagellin gene (flab) nested PCR which amplifies all Borrelia species. Borrelia was detected in 11% (22/205) of birds, with the highest prevalence in wild turkeys (16%; 5/31), followed by ruffed grouse (13%; 16/126) and American woodcock (3%; 1/35). Sequence analysis indicated only Borrelia burgdorferi was detected. All pheasants (n=13) were PCR negative. Our results support previous work indicating certain species of upland game birds may act as low-level reservoirs for Borrelia burgdorferi.

## WEST NILE VIRUS INFECTION IN RUFFED GROUSE (BONASA UMBELLUS) IN MICHIGAN, USA

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## Abstract

Ruffed grouse (Bonasa umbellus) are a medium sized game bird present throughout Canada, Alaska and the northern and eastern United States. Ruffed grouse experience natural ten-year fluctuations in populations which may be impacted by the availability of quality habitat, weather, predation, and disease. West Nile virus has been implicated in a reduction in ruffed grouse numbers in Pennsylvania. Ruffed grouse harvest and sightings were lower than anticipated in the Upper Great Lakes region in 2017, causing concern that the virus may be impacting populations in the midwestern United States. From August 2017 through November 2018, forty-six ruffed grouse that were found dead or observed behaving abnormally or emaciated prior to death were submitted to the Michigan Department of Natural Resources, Wildlife Disease Laboratory for postmortem examination. A necropsy was performed on each bird, tissues or blood feathers were tested for West Nile virus by polymerase chain reaction, and hearts from positive birds were examined via histopathology and immunohistochemistry. Nineteen of the 46 (41%) grouse were positive for West Nile virus on PCR. Sixteen of 18 (89%) PCR positive grouse showed heart lesions consistent with WNV infection and 7/18 (39%) had heart lesions positive by IHC. While many factors can influence ruffed grouse populations, West Nile virus is an additional stressor that can cause fatal infection in ruffed grouse. Further studies are needed to understand the potential impact of the virus on ruffed grouse populations.

# MORTALITY EVENT IN CANADA GEESE IN COLORADO ASSOCIATED WITH RIEMERELLA ANATIPESTIFER, AND PRESENTING AS VISCERAL GOUT

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### Abstract

During the winter of 2019, we documented a mortality event in Canada geese in northern Colorado. At least 100 dead Canada geese were identified, with rare reports of other species affected. Thirteen Canada geese were submitted to the Colorado Parks and Wildlife, wildlife health laboratory to determine cause of death. Preliminary diagnostics included gross necropsy, histopathology with special stains, aerobic culture of organs, lead testing, and PCR for avian influenza and avian paramyxoviruses. All 13 geese had gross and histologic evidence of severe visceral gout due to renal failure, as evidenced by extensive tubular necrosis in the kidneys. Tests for lead toxicosis, avian influenza virus, and avian paramyxoviruses were negative. Aerobic cultures demonstrated pure heavy growth of Riemerella anatipestifer (R. anatipestifer, formerly Pasteurella anatipestifer), as identified by MAL-DI-TOF, from lung and kidney of two affected geese. One affected goose demonstrated pure, heavy growth from lung and kidney of a Pasteurellaceae species that could not be further identified. Other bacterial isolates were of mixed or light growth and considered less significant. Riemerella anatipestifer is a well-described pathogen of waterfowl, although the presentation described here (visceral gout) is not typical for R.anatipestifer infections. Although R.anatipestifer was considered the most likely cause for this mortality event, the unusual presentation and inconsistent bacteriology results warranted further investigation.

# WEST NILE VIRUS AND TOXOPLASMA SEROLOGY, HEMOPARASITE PCR, AND NECROPSY RESULTS FROM WILD TURKEY (MELEAGRIS GALLOPAVO) IN MIDDLE TENNESSEE

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# Abstract

Wild turkey (Meleagris gallopavo) populations in three contiguous counties in middle Tennessee have declined due to unknown reasons. Current research is being conducted to determine recruitment, adult and juvenile survival, poult survival, and predation as potential factors in the decline. In addition disease testing is being conducted to determine if particular pathogens or disease complexes are a potential cause of population decline in wild turkeys. Turkey samples collected from 2016- 2018 were analyzed for infection or exposure for select pathogens including West Nile virus, Toxoplasma gondii and hemoparasites by serology or PCR. 103 (32.9%) of the 313 tested serum samples were seropositive for antibodies to West Nile virus. Toxoplasma antibodies were detected in 47 (20.2%) of 233 serum samples. Extracted DNA of liver tissue was tested for Plasmodium spp. and Haemoproteus sp. by PCR and DNA sequencing. Of the 215 tested samples, 119 (55.3%) were PCR and sequence positive for Haemoproteus spp. whereas all samples were PCR negative for Plasmodium sp. Histological examination of tissue sections from available birds, did not disclose lesions consistent with clinical infection of West Nile virus, T. gondii, or Haemoproteus. Other histological findings included Sarcocystis spp. cysts in muscle, lungworms consistent with Syngamus trachea, dermatitis, myocarditis, enteritis, and bacterial infection of the eye. Collectively, our results demonstrated turkeys are exposed to various pathogens and further research is needed to understand the clinical and population implications of these infections in wild turkeys.

# PREVIOUS USUTU VIRUS EXPOSURE PARTIALLY PROTECTS MAGPIES (PICA PICA) AGAINST WEST NILE VIRUS

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# Abstract

Currently at least five different flaviviruses are known to co-circulate in Spain and potentially other regions of Europe, namely West Nile virus (WNV) of lineage one and two, Usutu virus (USUV), Bagaza virus (BAGV) and Meaban virus. West Nile virus is a significant cause of mortality for many wild bird species in the North American continent, while in Europe wild bird deaths are usually sporadic, except for mass mortalities of Eurasian magpies due to WNV lineage 2 recorded in Greece in 2017/18. In contrast, Usutu virus a closely related flavivirus causes epizootics in wild and captive birds in Europe, but usually not in Eurasian magpies (Pica pica). The infection cycle of USUV in avian hosts and how sequential exposure of birds to co-cycling Flaviviruses affect infection outcome is virtually unknown. Our objective was to experimentally determine the susceptibility of Eurasian magpies to USUV, and explore how previous exposure to USUV would affect infection with lineage 1 WNV. None of the magpies survived, while only 22.2% (2/9) of those not previously infected with USUV did. Viremia and viral titer of WNV in feather pulps of previously USUV infection with USUV is not lethal for magpies, but partially protects them against a lethal challenge with WNV, although the mechanism is unclear. These results can be relevant for flavivirus ecology and contention.

#### AN INVESTIGATION OF LETHAL AND NON-LETHAL DOMOIC ACID EXPOSURE IN LOONS IN CALIFORNIA

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## Abstract

In 2017, an unusually large number of dead loons were found between Ventura and Los Angeles County in California, and live stranded loons were reported in large numbers at local wildlife rehabilitation centers. Concurrent to this increase in stranded birds, there was a large bloom of Pseudonitzschia with domoic acid (DA) toxin production. International Bird Rescue received 117 live loons between April 4 and May 30, 2017. Only nine loons showed typical clinical signs of DA intoxication at admission. Twelve birds (10.3%) died in the first 24 hours despite supportive care. Birds that survived the first few days quickly re-attained waterproof plumage, normal body weight and blood values, and 49 loons (41.9%) were successfully released; however, 56 loons (47.9%) never became releasable due to ongoing abnormal behavior or injuries related to beaching and were later euthanized. In order to characterize changes to the avian brain after exposure to DA, the brains of six loons euthanized due to long-term obtundation of up to 85 days were collected for histology. The CDFW Marine Wildlife and Veterinary Care and Research Center (MWVCRC) performed systematic necropsies on beachcast carcasses (n=14), including collection of samples for histopathologic examination. A subset (n=7) were analyzed for DA at University of California, Santa Cruz (UCSC). Seven out of seven birds examined at the MWVCRC tested positive for DA toxin at UCSC. Brains collected during gross necropsy at MWVCRC were also examined for acute exposure to DA. Gross and histological results were reviewed and compared to highlight the differences between lesions due to acute lethal DA exposure and lesions that developed over time subsequent to an acute non-lethal exposure. Currently, little is known about the effects of DA exposure in seabirds, and our study aims to fill gaps in this body of knowledge.

### SPECIES DIFFERENCES BETWEEN BISON, CATTLE, AND ELK IN SUSCEPTIBILITY TO BRUCELLA ABORTUS

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# Abstract

Within the United States, bison (Bison bison), elk (Cervus canadensis) and cattle (Bos taurus) are natural host for Brucella abortus. Established regulatory programs to control the disease in livestock hosts, partly for public health benefit, are currently being challenged by transmission of disease from wildlife reservoirs of brucellosis. In a series of studies, we characterized immunologic responses of bison, elk, and cattle to live attenuated vaccine strains and found that elk demonstrated significantly reduced cellular immune responses as compared to bison and cattle. After experimental infection of naïve animals in mid-gestation with the virulent B. abortus strain 2308, bison demonstrate the highest rates of abortion and infection, whereas elk demonstrate the lowest rate of abortion. In an effort to develop a challenge model that induces greater clinical symptoms, naïve pregnant elk (n=35) were randomly assigned to low (107 CFU) and high (108 CFU) conjunctival challenge doses administered early in the second trimester or at the beginning of the third trimester. Pregnant elk were euthanized after abortion or parturition and samples obtained for bacteriologic evaluation. Confirmation of successful challenge was by recovery of the challenge strain from conjunctival swabs at 5 days after challenge and demonstration of humoral responses to Brucella. Challenge dosage and stage of gestation did not influence (P>0.05) abortion rates, infection rates. and bacterial colonization. In comparison, cumulative data in naïve bison or cattle indicates abortion rates of 87% (66/76) and 46% (21/47), respectively. In comparison, an average abortion rate of 12.5% (5/40) is indicated by cumulative data in elk. Our data indicates that experimental challenge of elk with Brucella abortus does not cause abortion rates equivalent to effects seen in naïve bison or cattle. Increasing the challenge dosage or administering the challenge earlier in gestation does not appear to influence infection or abortion rates in elk.

# MATERNAL ANTIBODY PERSISTENCE IN NESTLINGS: FROM COMPARATIVE IMMUNOLOGY TO THE CONSERVATION OF ALBATROSSES

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# Abstract

Infectious diseases represent relatively neglected but potentially important threats to wild populations, especially in the current context of global change. Responses to those threats can rely on classical biomedical and veterinary approaches, but may also benefit from novel insight gained from studies focusing on the ecology and evolution of particular traits potentially playing key roles in host-pathogen interactions. The transfer of antibodies from breeding females to their offspring, and in particular the temporal persistence of such antibodies in nestlings, represents such traits. Although maternal antibodies have been known to persist in birds at detectable levels for less than three weeks after hatching, we predicted that longer temporal persistence may have evolved in species with an especially long chick-rearing period, notably because nestlings have to be protected against infectious agents during their time at the nest, when their immune system is still developing. Following our earlier results showing maternal antibodies to last more than several weeks post-hatching in the Cory's shearwater, we implemented transgenerational vaccination experiments in a set of other seabirds. We found much longer temporal persistence of maternal antibodies in the three Procellariiform (albatross and petrels) species considered than in the other seabird species. These results have potential implications in eco-epidemiology, veterinary science and biomedicine, but also for the conservation of threatened Procellariforms, as the vaccination of mothers could protect nestlings against pathogens to which they can be exposed to in the weeks after hatching. This is notably the case with avian cholera, due to Pasteurella multocida, which dramatically affects the survival of nestlings of threatened albatross species on Amsterdam Island, Indian Ocean, and for which we have been investigating the potential usefulness of a vaccination approach.

#### CLINICOPATHOLOGIC FINDINGS IN WHITE-TAILED DEER FAWNS INFECTED WITH RIFT VALLEY FEVER VIRUS

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# Abstract

Rift Valley fever (RVF) virus is a zoonotic, arthropod-borne Phlebovirus not currently in the US. The virus infects cattle, sheep and goats primarily through a mosquito-vertebrate host transmission cycle, and there is concern for incursion into susceptible host populations in the US. Thus, it is important to understand the susceptibility of white-tailed deer (WTD) and their potential epidemiologic role. Susceptibility of 5 mth-old WTD fawns to a wild-type strain of the virus (Kenyan 2006) has been shown and limited clinical and gross pathologic changes were described. We report susceptibility of 2 mth-old fawns to a different RVF virus strain (Egyptian isolate), along with additional clinical pathologic and histopathologic findings. Six fawns were inoculated subcutaneously with RVF virus strain ZH501 at three doses (103, 105, 107pfu/ml; two fawns each). Fawns were monitored and sampled for 3 to 10 days and euthanized as necessitated. Over the course of infection, oronasal and rectal swabs and serum were collected for virus isolation; whole blood was subjected to a CBC, liver enzyme analysis, and coagulation tests. Necropsy was performed on all animals. All developed clinical signs (one died; 3 required euthanasia). All were viremic on postinoculation day (PID) 1; viremia persisted until at least PID 5 in the fawn living to PID 10. All had rectal shedding of virus and half had oronasal shedding. At necropsy virus was detected in liver, spleen, lymph nodes, brain and eye. Clinical pathologic findings included leukopenia, prolonged prothrombin time and partial thromboplastin time, increased gamma-glutamyl transferase, alanine aminotransferase and bilirubin, decreased alkaline phosphatase and albumin, and severe lipidemia. At necropsy, hemorrhage and swollen livers were observed with massive hepatic degeneration and necrosis seen microscopically. Our work extends what is known about RVF in WTD in regards to age-based susceptibility, viral dose effects, and clinicopathologic findings.

# HOW THE ADDITION OF NESTING PLATFORMS INFLUENCE NEST PARASITISM BY (PROTOCALLIPHORA PARORUM) ON CORDILLERAN FLYCATCHERS (EMPIDONAX OCCIDENTALIS): CONSEQUENCES OF A NEWLY INTRODUCED PARASITE

Charles van Riper III

US Geological Survey & University of Arizona

# Abstract

The Cordilleran Flycatcher (Empidonax occidentalis) is a neotropical migrant bird species that is nest-site limited. The species is a nesting riparian obligate of the mountainous regions of the western US, and migrates to southern Mexico. The blow fly Protocalliphora parorum is known to only parasitize cavity-nesting birds and is not known to kill nestlings (Whitworth 1971). From 2007-2011 we studied breeding of the Cordilleran Flycatcher, adding nesting platforms in 2012 with a goal of enhancing breeding productivity. From 2012-14 Cordilleran Flycatcher reproductive success increased, and there were similar developmental periods and nest predation rates in natural and platform nests. However, in 2015 we recorded the first known case of nestling death caused by Protocalliphora parorum blow fly larvae, and this impact escalated each year through the end of our study in 2018. In this presentation we will discuss: 1) How development of a nesting platform enhanced nesting density and productivity of the Cordilleran Flycatcher in SW Colorado, 2) how the blow fly Protocalliphora parorum changed egg-laying location, thus greatly impacting a naive avian host, and (3) the consequences to Cordilleran Flycatcher productivity of a newly introduced wildlife disease factor. This information is important for understanding potential consequences of human assisted wildlife enhancement activities, especially as related to changing epidemiology of host/parasite interactions, especially when working to conserve neotropical migratory bird species of the Americas.

# ASSOCIATING ANATOMIC AND CLINICAL PATHOLOGY WITH BSAL CHYTRIDIOMYCOSIS IN THE ROUGH-SKINNED NEWT (TARICHA GRANULOSA)

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University of Tennessee, Knoxville UTIA Center for Wildlife Health

# Abstract

The anatomic pathology of Batrachochytrium salamandrivorans (Bsal) has been described in European newt species; however, the clinical pathology that occurs as the disease progresses remains an enigma. For a similar fungus, B. dendrobatidis (Bd), changes in blood electrolytes that lead to host paralysis and cardiac arrest have been reported. We hypothesized that newts experiencing Bsal chytridiomycosis would have similar changes in electrolytes, because like Bd, Bsal damages the epidermis hence likely affects skin osmoregulation – a key physiological process that influences blood chemistry. To test this hypothesis, we exposed a North American newt species (Taricha granulosa) to water baths containing one of four concentrations of Bsal zoospores (5x103, 5x104, 5x105, or 5x106) known to elicit disease (n = 5 newts per zoospore dose; n=2 controls) and monitored their condition for up to 52 days post-exposure. At euthanasia, blood was collected from the heart using heparinized capillary tubes, a blood smear was prepared, and a portion of whole blood was added to Natt Herrick's solution for total blood cell counts. The remaining blood was centrifuged and plasma collected for blood chemistry profiles and protein electrophoresis. The newts were preserved in formalin and processed for histological examination. Target lesions began to appear primarily on the heads of the newts within the 105 and 106 groups, and as early as 3 days post-exposure in the 106 group. Digits on the newts were red to dark brown with occasional target lesions. Microscopically, target lesions presented as partial to full depth crater-like areas of epidermal necrosis with intralesional thalli. Initial biochemical results revealed decreases in sodium, chloride, and potassium in terminal stages of the disease (5x106 dose) similar to Bd. Identifying biochemical changes associated with Bsal chytridiomycosis will help elucidate pathogenesis and be key to developing management strategies and exploring treatment options in susceptible species.

# EVIDENCE OF ACCUMULATION AND ELIMINATION OF INORGANIC CONTAMINANTS FROM THE LACHRYMAL SALT GLANDS OF LEATHERBACK SEA TURTLES (DERMOCHELYS CORIACEA)

Annie Page-Karjian<sup>1</sup>, Andreas Lehner<sup>2</sup>, John Buchweitz<sup>2</sup>, Justin Perrault<sup>3</sup>

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# Abstract

Plasma osmolalities of marine vertebrates are generally lower than the surrounding medium; therefore, marine organisms must cope with the osmoregulatory challenges of life in a salty environment. The salt glands serve to maintain osmotic and ionic homeostasis in a number of lower marine vertebrates. One marine reptile, the leatherback sea turtle (Dermochelys coriacea), ingests excessive amounts of salts due to their diet of gelatinous zooplankton. Outside of the normal osmoregulatory function of the salt gland, little research has been conducted on contaminant accumulation and excretion in this organ. Here, we established arsenic, cadmium, lead, mercury, and selenium concentrations in red blood cells (RBCs) and salt gland secretions (SGSs) of nesting leatherbacks. We also collected salt glands from different life stage classes of dead stranded leatherbacks from the western Atlantic Ocean to determine if inorganic contaminants accumulate in this organ. Using non-metric multidimensional scaling and regression analyses, we determined that RBC and SGS inorganic contaminant concentrations were not correlated. Additionally, RBCs showed significantly higher concentrations of these contaminants in comparison to SGSs, likely due to the affinity of inorganic contaminants for the heme group of RBCs. Lastly, we found that salt gland cadmium and mercury concentrations tended to increase with increasing curved carapace length (CCL) in stranded leatherbacks. Our results indicate that different physiological mechanisms determine the distribution of inorganic contaminants in blood and SGSs. Increases in salt gland contaminant concentrations with increasing CCL suggest this organ as a potential target for accumulation.

# SYNERGY OF GENETICS AND DIFFUSION DYNAMICS IN WHITE-TAILED DEER TO UNDERSTAND EPIDEMIOLOGY OF CHRONIC WASTING DISEASE

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## Abstract

Subpopulation structuring for a species determined by assessing microsatellite variation across a large geographic area can provide valuable information on assessing transmission dynamics of a disease. Combined with knowledge of environmental variables driving diffusion dynamics of a disease across a landscape, understanding disease epidemiology at broad spatial scales is possible. Here, we used genetic subpopulation structuring along with landscape conductivity pathways elucidated from disease samples collected during surveillance of chronic wasting disease (CWD) in white-tailed deer (Odocoileus virginianus) populations in the Mid-Atlantic region to understand potential drivers of disease across a landscape. We evaluated the relationship between genetic and resistance distances using maximum-likelihood population effects mixed models. We then assessed these resulting resistance surfaces, along with traditional assessment of barriers to gene flow, with ecological diffusion models to explore drivers of diffusion of CWD across the region. Regional surveillance efforts from 2012–2017 yielded over 30,000 white-tailed deer sampled for CWD with 2,222 of those tested being sampled for genetic analysis from 3 states. Gene flow was widespread but the extent and direction was influenced by forest cover, topography, and major highways. It appeared that the growth rate of CWD was lower in the core area of CWD infection compared to areas infection had established nearly a decade later. This is the first study to assess the synergy between gene flow in a region with on-going CWD surveillance to determine diffusion dynamics of a disease across the landscape. Disease progression through a landscape is not uniform and inclusion of landscape-specific drivers of diffusion is necessary for future projections of disease spread. Here we provide a framework for the integration of genetics and surveillance to better elucidate landscape-specific drivers of CWD spread.

# NEUROLOGICAL CLINICAL SIGNS ASSOCIATED WITH INFECTIOUS KERATOCONJUNCTIVITIS IN PYRENEAN CHAMOIS

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### Abstract

Infectious keratoconjunctivitis is an ocular disease that typically causes transient blindness in ruminants, but in wild species may eventually lead to eye perforation, traumatic accidents or starvation. In Caprinae, it is typically caused by Mycoplasma conjunctivae, which has a clear tropism to ocular structures and has not been isolated from other locations than the eyes or the nose. Therefore, some behavioral abnormalities such as circling movement described in severely IKC-affected chamois (Rupicapra pyrenaicaand Rupicapra rupicapra) or ibex (Capra ibex) have been exclusively related to blindness. A recent study based on molecular detection reported thatM. conjunctivaemay occasionally be located in external ear canals as occurs in other mycoplasma species. This study aims to examine the etiology and pathological abnormalities in nine severe IKC cases from Pyrenan chamois and mouflon (Ovis aries musimon), including among them thee chamois that exhibited horizontal nystagmus, involuntary lateral movement of the head and circling behavior. Preliminary results from CT scan and postmortem examinations include the presence of mucous content in the tympanic bulla in the three chamois that had neurological clinical signs. It suggests that severe IKC may occasionally be associated with a vestibular syndrome in wild Caprinae. These results will be presented together with histologic examinations, molecular detection of M. conjunctivaeand standard microbiological cultures from the eyes, nose, external ear canals, tympanic bulla, vestibulocochlear nerve and central nervous system on all the cases. This communication will clarify whether Mycoplasma conjunctivaeis able to invade middle or/and inner ear and cause a vestibular-like syndrome in severely IKC-affected chamois.

#### CHANGES IN CHRONIC WASTING DISEASE ECOLOGY AT ELK AT ROCKY MOUNTAIN NATIONAL PARK

Nathan Galloway<sup>1</sup>, Jenny Powers<sup>1</sup>, Ryan Monello<sup>1</sup>, Margaret Wild<sup>2</sup>

<sup>1</sup>National Park Service, <sup>2</sup>Washington State University

### Abstract

We conducted two key studies at Rocky Mountain National Park, Colorado, to investigate the population-level effects of chronic wasting disease (CWD) in elk with historically high densities (up to 110 elk/km2 on portions of the winter range). CWD was first detected in this population in 1981 and by the early 2000s half of the adult elk found dead tested positive for CWD. We estimated disease prevalence of ~13% (8-19%; n=136) in adult females in 2008. Additionally, we estimated that the population growth rate in female elk was flat ( $\lambda$ ~1.0) and that CWD can reduce adult female survival and decrease population growth of elk (Monello et al. 2014). In a subsequent study, we are investigating disease dynamics in the elk population and monitoring changes in disease transmission pressure associated with locally specific reduced elk density and increased elk dispersion. We have a preliminary estimate of prevalence for 2012-2016 of ~8.5% (4.6-13.3%; n=138). Results corroborate that CWD reduces adult female elk survival and this increased mortality decreases the population growth rate. Concurrent with our study, elk are re-distributing to lower elevations outside of the park, where CWD prevalence has always been lower, resulting in much lower densities within the park. The effects of this on CWD prevalence are unclear; movement may simply spatially dilute disease across the landscape or lower densities may reduce disease transmission.

# CHRONIC WASTING DISEASE: WISCONSIN'S PERSPECTIVE ON CONTINUING SURVEILLANCE AND MONITORING EFFORTS IN A WORLD OF ELECTRONIC DEER HARVEST REGISTRATION

Mandy Kamps, Tim Marien, Tami Ryan

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# Abstract

Chronic wasting disease (CWD) was first detected in Wisconsin in February 2002, from white-tailed deer (Odocoileus virginianus) that were harvested during the 2001 deer hunting season. At that time, Wisconsin had in-person registration for deer hunting, which allowed for more convenient CWD sample collection. However, in 2015, state regulations changed, and in-person registration turned into electronic registration. With this came a need to evaluate CWD sample collection methods. Objectives were to adjust existing or identify new methods so surveillance plans could still be achieved. Hunter service request testing has always and continues to be available to deer hunters statewide. Self-sample CWD kits were piloted in 2016 and continued in 2017 and 2018. In 2015 self-service kiosks were made available to hunters as a pilot test: kiosks had all supplies necessary for hunters to submit a deer head and complete a datasheet for CWD testing. Kiosks became popular with deer hunters and in 2018, a new Adopt-a-Kiosk initiative was available for individuals/organizations to assist with CWD surveillance. CWD surveillance permits were created for use on private lands, and in 2018 they were also made available for use on public lands, within specific surveillance areas. Overall, these CWD sample collection methods have been effective in increasing sample volume in a world of electronic registration and reaching annual surveillance plans. In 2017 and 2018 total submissions were about 2,000 samples above the annual surveillance plans, however, each sample collection method has pros and cons and some (eg. kiosks) are more effective than others (eg. self-sample kits). Sufficient education and outreach are needed to inform hunters of CWD sample submission options/methods. It is imperative to plan well in advance of deer hunting seasons and determine which sample collection methods are best to achieve sampling and surveillance goals.

#### DEER MOVEMENT IN MINNESOTA AND THE POTENTIAL TO SPREAD CHRONIC WASTING DISEASE

Kelsie LaSharr, Chris Jennelle, Michelle Carstensen, Lou Cornicelli

Minnesota Department of Natural Resources

### Abstract

In 2018, the Minnesota Department of Natural Resources (MNDNR) began a multi-year study to analyze whitetailed deer (Odocoileus virginianus) movement in and around a chronic wasting disease (CWD) management zone in southeastern Minnesota to help predict pathways of CWD movement and support future disease management decisions. We believe CWD can be spread long distances through natural deer movements; juvenile deer (<1 year old) disperse from their natal to adult (> 1 year old) home ranges and males make excursion movements during the fall rut. To further formalize this expectation, we hypothesized that juvenile males have a higher probability of dispersing from their natal range and will travel longer distances than juvenile females. In March 2018, we placed Iridium GPS collars on 109 deer (34 juvenile females, 49 juvenile males, 1 adult female, and 25 adult males). During the first year of the study, mortalities (n=29) and collar failures (n=56) reduced the number of animals available for analysis. We used linear movements of 4km or more to delineate a spring dispersal by juveniles or significant excursion by adults. In spring 2018, 45% (n=9/20) of juvenile females and 32% (n=10/31) of juvenile males dispersed from their natal range. On average females traveled 9.2km, males traveled 16km. In fall 2018, 36% (n=4/11) of females and 15% (n=4/26) of males conducted excursions from their adult home range, traveling an average of 6km and 19km respectively. In February 2019, MNDNR conducted a second round of captures and 64 additional juvenile deer were recruited into the study (39 females and 25 males). Results from the spring dispersal will be presented at the conference.

#### **BRUCELLOSIS IN ELK IN IDAHO, 1998-2018**

Mark Drew

Idaho Department of Fish and Game

#### Abstract

Brucellosis due to Brucella abortus is a bacterial disease of ruminants and is associated with abortions, stillbirths, and impaired reproductive performance. Brucellosis in the Greater Yellowstone Ecosystem has been of concern for over 100 years with elk and bison acting as reservoirs of the disease for cattle. Idaho initially identified brucellosis in elk in 1998. Since that time, surveillance and management actions were implemented to minimize the impact of brucellosis in elk and spillover from elk to cattle. From 1998-2018, 4461 samples from elk harvested by hunters were received with 3490 testable samples. A battery of tests including BAPA, standard plate or tube, FPA and CF were used to determine exposure status. Only 118 elk are seropositive for brucellosis (2.6% seroprevalence). In the same period, 3800 elk were captured for management purposes with 178 seropositive animals (5.0% seroprevalence). Seroprevalence appears to be stable and the geographic distribution remains largely within the DSA in eastern Idaho. The finding of a small number of seropositive elk outside the DSA may indicate some exposure to brucellosis or more likely, Yersinia spp. To delineate the boundary of the brucellosis surveillance area (DSA) for USDA and ISDA, elk surveillance represents the least expensive and widest distribution of elk to define the known distribution of brucellosis exposure in elk in Idaho and the area in which there is a possibility of elk-cattle interactions during the risk period. Management actions include efforts to keep elk and cattle separated in winter during the primary transmission period using hazing, directed feeding, and fencing. Despite these efforts, elk to cattle transmission has occurred in five cattle herds resulting in quarantine and/or depopulation of the cattle. Prevention of brucellosis transmission from elk to cattle will require continual surveillance, temporal-spatial separation during winter months, and possible reduction of elk numbers.

# A HIGH MORTALITY OUTBREAK OF MYCOPLASMA BOVIS IN WYOMING PRONGHORN (ANTILOCAPRA AMERICANA)

Jennifer Malmberg<sup>1</sup>, Terry Creekmore<sup>2</sup>, Erika Peckham<sup>2</sup>, Marguerite Johnson<sup>1</sup>, Halycon Killion<sup>1</sup>, Madison Vance<sup>1</sup>, Donal O'Toole<sup>1</sup>, Kerry Sondgeroth<sup>1</sup>

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## Abstract

Mycoplasma bovis (M. bovis) is an economically important pathogen of cattle best known as a component of polymicrobial respiratory disease and an agent of mastitis and polyarthritis. Recently, M. bovis emerged as a cause of high morbidity and mortality in ranched North American bison, raising concern for emergent strains with high virulence. In 2019, M. bovis was identified as the primary cause of a regional outbreak of fibrinous pleuropneumonia in pronghorn near Gillette, WY. Of at least 60 animals found dead between February and April, M. bovis was detected in 9 by PCR, immunohistochemistry (IHC), or both. M. bovis has not been previously reported in pronghorn, and reports in free-ranging wildlife are limited to rare, isolated events or infections. In the current study, we characterized the pathology and genetics of M. bovis infection in pronghorn, and compared our findings to those documented in cattle and bison. We report that M. bovis causes severe fibrinosuppurative pleuropneumonia as a sole or primary pathogen in pronghorn, similar to infections in bison. Whole genome sequencing of M. bovis from pronghorn revealed a deletion of the alcohol dehydrogenase-1 gene (adh-1), a target of multilocus sequencing typing (MLST) in cattle and bison. We queried the Wyoming State Veterinary Laboratory's diagnostic database for historic cases of pronghorn mortalities and identified 12 cases of pneumonia and 7 additional cases with non-pneumonic lungs over the past 12 years. All were negative for M. bovis on retrospective evaluation by IHC and PCR, suggesting M. bovis emergence in pronghorn is a recent event. We conclude that M. bovis has continued to expand in disease expression and host range in recent years, and should be considered as a differential for pneumonia in free-ranging ungulates across North America.

#### RABIES VIRUSES ORIGINATED AMONG DIVERSE NEW WORLD WILDLIFE

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<sup>1</sup>LYSSA LLC, <sup>2</sup>Animal and Plant Health Agency

### Abstract

A global 2030 program, for the elimination of human rabies caused by dogs, is a priority for the World Health Organization. As such, given renewed proximate efforts, in context of disease burden and extensive virtual reach, re-examination of essential underpinnings for ultimate occurrence of this high-consequence zoonosis is warranted. Despite descriptions as one of the oldest infectious diseases, precisely when, where and how rabies originated is unknown. More than 16 different lyssaviruses are described, but only rabies virus has been documented throughout the New World. Based upon a trans-disciplinary approach encompassing data from multiple fields, the most parsimonious explanation for recent distributions of lyssaviruses is dependent upon the survival of discrete host-virus assemblages following major mass extinctions over hundreds of millions of years. Major concomitant biological bottlenecks and emancipation involved a series of inter-related events, including: early evolution of an RNA-dependent, RNA polymerase and basic definition of the Mononegavirales, with rise of the rhabdoviruses in multi-cellular organisms; accessibility to a central nervous system, as a critical niche; leaps from arthropods to other highly vagile animals; exploitation of homeothermy, sociality and teeth as primary viral conduits towards vertebrate perpetuation; ancestral mammalian origins in Gondwana; adaptation to bats; radiation of rabies viruses among the Phyllostomidae, with spillover and shifts to Carnivora in the New World; Holarctic distribution of canids; advance of Homo migrations out of Africa; bi-directional translocations across Beringia; canine domestication in Asia; flourishing of agriculture, urbanization and human civilization networks; and enhanced cosmopolitan spread of canine rabies and emergence among meso-carnivores, with European re-introduction into the Americas during colonization. While canine rabies elimination is a highly desirable and achievable public health goal, rabies eradication is not possible at the current time, especially considering the long-term history of pathogen entrenchment among diverse, gregarious warm-blooded hosts, offering opportunities for wildlife health professionals.

## INTEGRATION OF WILDLIFE AND ENVIRONMENTAL HEALTH INTO A ONE HEALTH APPROACH

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## Abstract

Loss of biodiversity, habitat fragmentation, pollution, and other environmental threats are degrading natural environments, which jeopardizes the range of ecosystem services that support all life on this planet. These changes, among others, are also driving the emergence of infectious diseases with negative health outcomes for humans, animals (livestock, wildlife, and other species), and our shared environment. Historically, interventions aimed at human and livestock health issues did not always integrate wildlife or environmental health as part of the solutions, which has resulted in unintended negative consequences. One Health recognizes the interdependence of humans, animals, and their shared environment, and provides a conceptual framework for developing interventions that optimize outcomes for human, animal, and environmental health. However, we argue there is a need to clearly articulate the core values, goals, and objectives of One Health for all relevant sectors in order to maximize synergies for communication, coordination, collaboration, and ultimately for joint actions on disease control and prevention. We also propose that the application of systems and harm reduction approaches; focusing on the socio-economic and environmental determinants of health, or the base of the "One Health Impact Pyramid"; and ensuring good governance and effective leadership will maximize the opportunities to develop "win-win" solutions to global health and environmental challenges. These solutions we believe will assist in fully integrating wildlife and environmental health into One Health, and propel it forward to reach its full potential by truly optimizing health outcomes for all.

# "THEY'RE ALWAYS THERE": CHARACTERIZING RAT EXPOSURE AND ITS CONSEQUENCES AMONG RESIDENTS OF AN IMPOVERISHED, INNER-CITY NEIGHBORHOOD IN VANCOUVER, CANADA

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<sup>1</sup>Animal Health Centre, British Columbia Ministry of Agriculture, <sup>2</sup>University of British Columbia, <sup>3</sup>Public Health Agency of Canada, <sup>4</sup>Centers for Disease Control and Prevention

# Abstract

Rat infestations are common in impoverished, inner-city neighborhoods. However, there has been little research into the nature and consequences of rat exposure among residents of these areas. The study objective was to characterize exposure to rats among residents of Vancouver's Downtown Eastside (DTES, known as Canada's poorest postal code), as well as the risk factors for exposure and its physical and mental health impacts. Participants (N= 202) provided blood samples and answered a questionnaire regarding the frequency and nature of their exposure to rats, sociodemographic characteristics, drug-use, and other behaviors. Blood samples were tested for exposure to the rat-associated bacterial zoonoses Leptospira interrogans and Bartonella tribocorum (known to be circulating in DTES rat populations). Logistic regression was used to identify risk factors for rat exposure. Semi-structured interviews regarding experiences with, perceptions of, and feelings towards rats were synthesized using thematic analysis. Six of 202 (3.0%) residents were exposed to B. tribocorum. There was no serological evidence of exposure to L. interrogans. Residents reported frequent, close encounters with rats, and exposure was associated with injection drug use and outdoor income-generating activities (e.g., drug dealing). Rat exposure elicited a number of negative psychological reactions, including disgust, fear, avoidance, sleep disturbances, and feelings of feelings of anger, sadness, depression, and marginalization. Members of impoverished, inner-city communities have significant contact with rats. This contact is associated with specific behaviors that could be mitigated through harm reduction techniques. Although contact with rats could pose a physical health risk through the transmission of zoonotic diseases, the mental health impact of living with rats is an important but underappreciated public health risk.

# URBAN MUSKRATS AS AN INTERMEDIATE HOST OF ECHINOCOCCUS MULTILOCULARIS IN ALBERTA, CANADA

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## Abstract

Background: Urban wildlife are increasingly recognized as a source of zoonotic pathogens, which is concerning given the rates of global urbanization. In urban and rural areas of Alberta, Canada, there are increasing reports of liver infections in humans and domestic dogs with the zoonotic tapeworm parasite Echinococcus multi-locularis (EM), which seems to be maintained in urban coyotes and foxes. Normally, EM cycles through predator-prey relationships between canids and rodents. Incidental ingestion of eggs can result in alveolar echinococcosis in humans and domestic dogs, often with severe, fatal disease in the latter. Small rodents (i.e., Microtus pennsylvanicus, Peromyscus maniculatus) were reported to act as intermediate hosts in urban Alberta. In Europe, muskrats (Ondatra zibethicus) are established intermediate hosts; however, the role of this urban-adapted, semi-aquatic rodent in the transmission of EM is unknown in North America. As part of ongoing wildlife disease surveillance, we received 93 muskrats trapped in urban marshes of Calgary for diagnostic investigation at the Canadian Wildlife Health Cooperative (Alberta region).

Objectives and Methods: 1) identify EM infections in urban muskrats using macroscopic assessment and histopathology; and 2) assess demographic risk factors.

Results: Liver lesions consistent with alveolar echinococcosis were present in 23% (21/93) of muskrats and many were fertile infections containing protoscoleces. Affected organs also included mesentery, spleen, reproductive organs, omentum, and kidney. The average number of liver cysts was 2 (range 1-4). No demographic risk factors were significantly associated with infection (sex, body condition, body mass; p>0.05). Conclusions: The close association between urban wildlife, people and their pets create opportunities for the direct and indirect transmission of pathogens, including EM. These results show that muskrats are competent intermediate hosts and may play an important role in the ecology of this locally-emerging zoonotic pathogen, which may have implications for urban disease surveillance and control initiatives.

### SARCOPTIC MANGE IN WILD FOXES IN HUMAN-DOMINATED LANDSCAPES, CHILE

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# Abstract

Sarcoptic mange is a parasitosis occurring worldwide caused by the highly contagious mite Sarcoptes scabiei, which affects human, domestic animals and wildlife. Mange has been described from a range of wildlife species including carnivores. In some cases, it has shown to have catastrophic consequences for wildlife populations. Our goal was to describe the epidemiological features of mange in Andean (Lycalopex culpaeus) and South American grey foxes (L. griseus) thriving in the highly anthropized environments of central Chile. From 2015-2018, we captured 64 Andean foxes and, using passive surveillance, we inspected and/or necropsied 24 additional Andean and 15 grey foxes. Mange lesions were confirmed by visualization of the mites in skin scrapes and histologic sections from necropsied animals. Serum samples were analyzed by ELISA. Spatial analyses were conducted to study potential risk factors of infection in wild-caught foxes. External lesions were observed in 7 (11%) wild-caught and in 8 (33%) passively surveyed Andean foxes and in 1 (6%) grey fox. Seroprevalence was 57%, 79% and 57% in each the previously mentioned groups. Macroscopic lesions consisted mostly of alopecia and crusts with the tail, legs and nose most frequently affected. Histologically, the epidermis was variably thickened with marked hyperkeratosis, corneal pustules, serocellular crusts and superficial dermal inflammation. Spatial analyses showed that mangy foxes were captured significantly closer to human settlements than foxes without lesions. A similar tendency was also observed for seropositive individuals. Altogether, results indicate that scabies in wild foxes is more frequent than previously recognized. Contact with domestic animals may be a risk factor for infection, or, alternatively, diseased foxes may approach humans in the search for food.

# RED FOXES (VULPES VULPES) AND COYOTES (CANIS LATRANS) IN AN URBAN LANDSCAPE: PREVALENCE AND RISK FACTORS FOR HEARTWORM

Meghan Pluemer<sup>1</sup>, Shelli Dubay<sup>1</sup>, David Drake<sup>2</sup>, Shawn Crimmins<sup>1</sup>, Tessa Veverka<sup>1</sup>, Holly Hovanec<sup>2</sup>, Miranda Torkelson<sup>2</sup>, Marcus Mueller<sup>2</sup>

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## Abstract

Urbanized landscapes pose novel disease risks for humans, their domestic pets, and urban wildlife. Canine heartworm is a mosquito-borne disease caused by the nematode Dirofilaria immitis and can affect pulmonary and respiratory function when the parasites inhabit the heart and arteries. Coyotes (Canis latrans) with intense infections have shown reduced activity, decreased fur quality, and reduced reproductive success. Additionally, the abundance of the parasite may differ by physical location, amount of precipitation, and abundance of mosquito vectors. The objectives of our study were: 1) identify the prevalence of canine heartworm in urban red foxes (Vulpes vulpes) and coyotes, and 2) determine if specific land cover characteristics could explain the presence or absence of heartworm. We trapped and radio-collared fifteen red foxes and fourteen coyotes in the urban landscape of Madison, Wisconsin. We used the SNAP 4Dx Plus (IDEXX Laboratories, Westbrook, ME) test, a rapid qualitative ELISA test that uses EDTA whole blood, to detect heartworm (Dirofilaria immitis) antigen. Geographic Information System was used to create a map of the study area based on National Land Cover Database. Selection and avoidance were calculated for nine radio-collared coyotes and eight radio-collared foxes in the study area and compared to heartworm infection. None of the foxes were infected with heartworm, while five (35.7%) coyotes were infected. Using Fisher Exact Probability Tests, we found that coyotes had a significantly higher prevalence of canine heartworm (p-value=0.02) than foxes. In general, red foxes selected for developed areas while coyotes selected for natural areas. Heartworm infection was positively correlated with selection for woody wetland cover in this study. All heartworm-infected coyotes resided within the UW-Madison Arboretum. Understanding the prevalence of disease in urban canid populations is important because diseases that affect urban canids cause morbidity and mortality and are transmissible to domestic dogs, and vice versa.

# ELUCIDATING THE ECOLOGY OF LEPTOSPIRA INTERROGANS IN URBAN NORWAY RATS (RATTUS NORVEGICUS)

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## Abstract

Norway rats (Rattus norvegicus) are the reservoir for zoonotic Leptospira interrogans in cities. Transmission among rats was thought to be a consequence of random environmental exposure; however, recent evidence has suggested that interactions among rats and/or the urban landscape may influence L. interrogans ecology. Specifically, age and bite wounds have been associated with L. interrogans carriage and there is unexplained heterogeneity in pathogen distribution. The study objective was to investigate whether salivary shedding explains the link between bite wounds and infection; to determine whether features of the urban environment influence L. interrogans distribution; and to compare/contrast L. interrogans ecology in Vancouver, Canada, and Salvador, Brazil. For rats trapped in Vancouver (N = 1,303) and Salvador (N = 433), urine/kidney and oral swabs were tested for L. interrogans by PCR and demographic/morphometric data were collected. In Vancouver, each city block was characterized using a 58 item questionnaire. Multi-level models were used to identify environmental predictors of infection and to compare/contrast L. interrogans ecology between cities. There was no evidence of salivary shedding of L. interrogans and no significant associations between environmental features and L. interrogans carriage. The prevalence of L. interrogans was greater in Salvador (79%) than Vancouver (12%), but distribution was more heterogeneous in Vancouver. In both cities, age and bite wounds increased the odds of L. interrogans carriage, but rats in Salvador were more likely to leave the nest infected. The link between L. interrogans infection and bite wounds likely reflects urine exposure during aggressive encounters, rather than direct salivary transmission, and the determinants of L. interrogans infection are consistent between cities. Rat social structures and interactions, rather than features of the urban environment, appear to be the strongest predictors of L. interogans carriage. This information is critical for developing pest control strategies to decrease rat-associated zoonotic risks.

#### **RACCOONS, FERAL CATS AND HUMANS: URBANIZATION EFFECTS ON WILDLIFE HEALTH AND ZOONOSES**

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#### Abstract

Human activities promote novel human-domestic animal-wildlife interactions enhancing pathogen transmission. Raccoons (Procyon lotor) are highly adaptable and opportunistic, benefitting from anthropogenic resources, including provisioned food at feral cat feeding stations within TNR colonies. Prompted by a concurrent raccoon rabies and canine distemper epizootic on a barrier island (Jekyll Island [JK], Georgia), this study was developed to investigate pathogen dynamics of raccoons under different levels of anthropogenic influence. Raccoons (n=86) were sampled from 2016-2018 from various sites on JK and nearby mainland at Wormsloe Historic Site (WHS). JK is a state park, a third of which is urbanized. Raccoons were trapped at TNR feral cat stations. WHS is a natural preserve with no urbanization or feral cats, adjacent to low-development and cattle ranching. We hypothesized that raccoons from WHS would have a lower pathogen prevalence and diversity than those from JK. Raccoons were tested for antibodies to canine distemper virus (CDV), canine parvovirus (CPV), and Toxoplasma gondii, infection with Babesia, and shedding of Salmonella. We also compared body condition index (BCI), body condition score (BCS), ectoparasite diversity/burden. Lastly, we compared raccoon density in JK sites with/ without TNR feeding sites. The prevalence of Salmonellashedding and serotype diversity, T. gondiiand Babesias.s. spp. was higher at WHS. In contrast, the prevalence of CDV, Babesiamicroti-like spp., BCI, BCS, ectoparasite burden and diversity were significantly higher at JK. JK had a higher number of zoonotic serotypes (e.g. Anatum, Montevideo, Muenchen), while at WHS there was a higher number of serotypes associated with livestock (e.g. Bovismorbificans, Give). On JK, sites with TNR feeding stations had a higher density of raccoons, and higher diversity and density of cats and other wildlife (e.g. grey fox, opossum, deer) than at sites without feeding stations. These data highlight the complexity of human-induced factors on pathogen transmission.

# ENDEMIC SKUNK AMDOPARVOVIRUS IN FREE-RANGING STRIPED SKUNKS (MEPHITIS MEPHITIS) IN CALIFORNIA

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## Abstract

Amdoparvovirus is a little known genus. It includes multiple newly discovered species such as the Skunk Amdoparvovirus, as well as Aleutian disease virus which causes significant health impacts in farmed mink worldwide. In 2010-2013, an outbreak of a fatal Amdoparvovirus presumed to be Aleutian disease was documented in free-ranging striped skunks (Mephitis mephitis) from the San Francisco Bay Area of California. To characterize the geographic distribution and number of infections of this virus, we collected skunk samples throughout California and tested for Amdoparvovirus viremia. Amdoparvovirus viral DNA was detected in 64.8% of sampled skunks (140/216), and viremia-positive skunks were distributed widely throughout the state, ranging from as far north as Humboldt County and south to San Diego County. No positives were detected in 38 fishers sampled. The earliest year of test-positive skunks was 2004, indicating the virus was present undetected in the population prior to the 2010-13 outbreak. No significant spatial or temporal clustering of infection was detected. Both healthy and clinically ill animals tested positive for Amdoparvovirus DNA. Histopathologic evaluation of a subset of samples from clinically ill skunks indicated that the presence of bronchopneumonia, or one or more lesions (any type) suggestive of Amdoparvovirus infection were statistically significant predictors of a positive PCR result. Given the widespread geographical distribution and lack of a clear epizootic center, our results suggest a skunk-specific Amdoparvovirus strain or species is endemic in the skunk population of California. Our data support the possibility of skunks as both a reservoir and target host: skunks' ubiquitous presence across rural and urban habitats and subclinical carriers pose a risk to susceptible domestic and wild species. Continued investigation of Amdoparvoviruses in skunks is warranted as well as testing of additional at-risk species including those of conservation concern such as fishers (Pekania pennanti) and Pacific martens (Martes caurina).

# CASES OF SUSPECTED AND CONFIRMED CARBOFURAN TOXICOSIS IN RAPTORS IN THE MID-ATLANTIC UNITED STATES

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## Abstract

Carbofuran (C12H15NO3) is a highly toxic neurotoxic pesticide that acts by inhibiting the cholinesterase enzymes, resulting in an accumulation of acetylcholine and subsequent overstimulation of the parasympathetic nervous system, and to some degree, the central nervous system. This overstimulation in birds is manifested in clinical signs affecting the eyes (miosis, ptosis, lacrimation, exophthalmia, blindness), neuromuscular system (piloerection, opisthotonos, hyperexcitability, lethargy, ataxia, paresis, convulsions), gastrointestinal system (defecation, diarrhea, vomiting), respiratory system (dyspnea, tachypnea), and in severe cases may result in death. Birds are not able to metabolize carbamates well, making them susceptible to both primary and secondary intoxication, so convulsions and death are not uncommon sequelae. Although effectively banned by the EPA in 2009, carbofuran continues to be used by landowners as a means of baiting and eliminating 'nuisance' wildlife species, often resulting in toxicity in non-target species. Nearly every year since 2007, Tri-State Bird Rescue & Research, Inc., a wild avian rehabilitation center in Newark, DE, has received raptors (mainly bald eagles (Halieetus leucocephalus)) with clinical signs of carbamate toxicity. A review of these cases demonstrates that rapid recognition and treatment can lead to full recovery of affected birds, and rapid removal of carcass remains may prevent other animals from becoming intoxicated. Similarly, rapid collection of blood and ingesta, carcasses, and other evidence is vital as carbamates break down rapidly and rarely concentrate in tissues. As a result, laboratory confirmation of exposure is often based on a combination of decreased cholinesterase activity in the affected animal and evidence of carbamates in the ingesta.

# INVESTIGATION OF FACTORS ASSOCIATED WITH PERSISTENCE OF AVIAN INFLUENZA VIRUSES IN THE ENVIRONMENT

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# Abstract

The maintenance of infectious influenza A viruses (IAVs) in North American wetlands may influence the indirect transmission of viruses among wild birds and subsequent dispersal through space and time. It is unknown if IAVs remain infectious in North American wetlands for extended periods, seeding outbreaks in wild birds across seasons or in free-ranging poultry after migratory birds have departed from wintering areas. As such, research to understand the persistence of infectious IAVs in the environment has been identified as a priority for improving epidemiological models of transmission. In this study, we conducted a series of related field and lab experiments to test if IAVs shed by wild birds can be detected, cultured, and transmitted to live captive birds following an extended period (e.g., several months) under both laboratory-controlled and field conditions at ecologically relevant doses. Furthermore, we assessed how environmental factors, such as water chemistry and temperature, relate to virus persistence. Results provide information on the viability of IAVs in North American wetlands which may be used to evaluate the risk of persistence of foreign-origin and/or highly pathogenic influenza A viruses in the environment. Furthermore, information relating water temperature, chemistry, and genomic characteristics of viruses to viral persistence in the environment may be used to forecast the viability of foreign-origin and/or highly pathogenic influenza A viruses in specific wetland complexes or regions of the United States and Canada. Finally, data may be used to support prioritization of specific wetlands or wetland complexes for IAV surveillance sampling for viruses of economic interest or of concern to human/domestic animal health.

# THE CHICKEN OR THE EGG? THE INTERPLAY OF DISEASE AND CLIMATE CHANGE ON WILDLIFE POPULATIONS

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### Abstract

Climate and other environmental change has implications for the emergence and spread of many pathogens and alternations to infectious disease processes. These implications pose a dilemma for agencies such as the National Park Service (NPS), which is charged with conserving natural resources while also providing for public enjoyment and protection of human health. The complexity of infectious disease ecology, range of projected environmental changes, and diversity of responses of disease-causing pathogens, vectors, and hosts will make it particularly challenging for managers to accurately predict outbreaks to protect resources and human health. Careful examination of the state of the knowledge and recognition of the knowledge gaps for any given system are integral to selecting an appropriate strategy for disease management. Warmer temperatures and more variable weather patterns can impact disease organisms, particularly those transmitted by arthropod vectors, in multiple ways. At the same time, vector borne diseases are dramatically influenced by ecological factors strongly altered by human settlement and development. We examine tick-borne, flea-borne, and mosquito-borne diseases that pose risks to National Park resources and visitors, and discuss the multiple factors driving both disease occurrence and impacts. We argue that issues of climate change, loss of connectivity, biodiversity loss, and species introductions, including pathogens, must be examined in concert for the best management outcomes. Managers are encouraged to address infectious disease from an ecosystem processes standpoint, and evaluate management options from the disease impact perspective rather than based on historic categorization of a specific organism or microbe.

