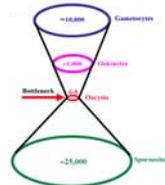## Past

- *Plasmodium falciparum* and *P. vivax* are co-endemic in Asia.
- We explored transmission of variants through the mosquito.
- Haplotypes were compared between patient blood and patient blood that was membrane fed to mosquitoes.

Transmission through the mosquito

Wang and Jacobs-Lorena. Trends Biotechnol. 2013
UNC INFECTIOUS DISEASE EPIDEMIOLOGY AND ECOLOGY https://www.med.unc.edu/infdis/ideel/

Past

- *Plasmodium falciparum* and *P. vivax* are co-endemic in Asia.
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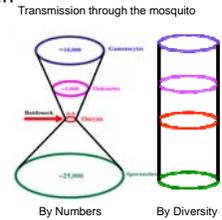- Bioinformatics pipeline SeekDeep*

Transmission through the mosquito

By Numbers   By Diversity

*Hathaway NJ, Parobek CM, Juliano JJ and Bailey JA. (2018) Nucleic Acids Research, doi:10.1093/nar/gkx1201
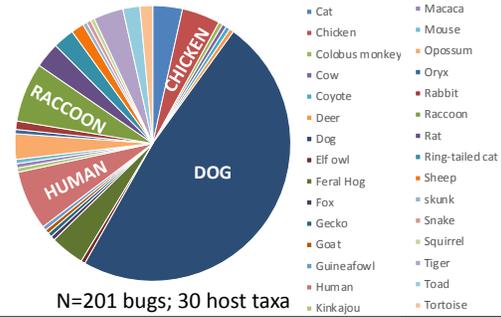
Present

- Dr. Sarah Hamer's Lab focuses on ecology and epidemiology of disease at the interface of wildlife, domestic animal, and human populations.
- The vector of interest is *Triatoma*, which carries *Trypanosoma cruzi*
- We look at identifying bloodmeal hosts using a mitochondrial gene in Amplicon Deep Sequencing.
- Bioinformatics pipeline – SeekDeep* + NCBI BLAST

*Hathaway NJ, Parobek CM, Juliano JJ and Bailey JA. (2018) Nucleic Acids Research, doi:10.1093/nar/gkx1201

Present

- Bloodmeal analysis of triatomines: status quo Sanger sequencing identifies only single host per bug

Cat	Macaca
Chicken	Mouse
Colobus monkey	Opossum
Cow	Oryx
Coyote	Rabbit
Deer	Raccoon
Dog	Rat
Elf owl	Ring-tailed cat
Feral Hog	Sheep
Fox	skunk
Gecko	Snake
Goat	Squirrel
Guineafowl	Tiger
Human	Toad
Kinkajou	Tortoise

CHICKEN   RACCOON   HUMAN   DOG

N=201 bugs; 30 host taxa

Present

- Amplicon deep sequencing (Illumina MiSeq) reveals host community

Bugs feeding on multiple hosts!
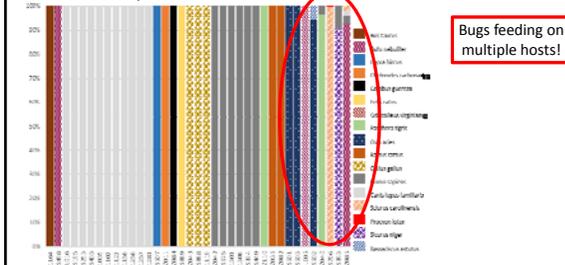
N=38 bugs; 17 host taxa

Future

- Following our interest in spread of disease, we look at migratory birds and their ectoparasites.
- Applying NGS techniques to examine pathogen carriage.

National Audubon Society
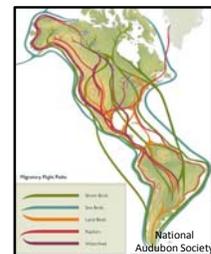
Byron R. Buckley, M.S.
Genetic Assessment of Northern Bobwhite Immune System
Texas Tech University

Decline

- Range wide decline noted by 1940's
- Texas population
  - 5.8% decline 1999-2009
    - 2.8% decline in Texas panhandle region
  - WHY?

BBS Trend Estimates 1966-2003

We are still working on the causes

- Habitat?
- Human?
- Predation?
- Reproduction failure?
- Disease?*

Immune System Analysis

Ecoregions of Texas

Map credit: http://www.tpwd.state.tx.us/

- Toll like receptors
  - Correlations in survival?
  - Population numbers?
- Population diversity
  - Microsats
  - Immune genes

- 2000 samples across 1.2 million acres

Stefano Catalano

Multi−host Spectrum of *Schistosoma* species/ hybrids and epidemiological role of humans, livestock, wild mammals and snails in West Africa

Royal Veterinary College

PhD Student at the Royal Veterinary College

Multi-host spectrum of *Schistosoma* species/hybrids and epidemiological role of humans, livestock, wild mammals and snails in West Africa

credit E. Léger

Research Interests & Past Experiences

Parasitology & One Health applied to:
1. Wildlife conservation and ecosystem health
2. Diseases at human-wildlife interface

NGS Setup Protocols and RADseq Data

Andrews et al. 2016 *Nature Reviews*

credit Sanger Institute

Liliane Cavalcante
Characterization of wild felids Virome
Federal University of Rio de Janeiro

Research - Virome

Leopardus Tigrinus (Northern tiger cat)

Panthera Onca (Jaguar)

Panthera Leo (Lion)

Leopardus Pardalis (Ocelot)

Puma Concolor (Montain lion)

Panthera Tigris (Tiger)

Methods
- Captive species (From Rio de Janeiro Zoo)
- Virus DNA/RNA extraction
- Illumina sequencing
- Data processing:
  - Filter low quality and small size reads (Sickle).
  - Filtering host reads (bwa/samtools)
  - Blastx against vertebrate virus data bank
  - Blastx against nr/nt data bank
  - Contigs assembling (Geneious)

Liliane Cavalcante - PhD Student
Federal University of Rio de Janeiro, Brazil

Goals

- Identify the major circulating viruses in wild cat species.
- Characterize viruses by sequencing the entire genome.
- Check the correlation of the presence of viruses and clinical signs.

What we want is...

- Optimize the pipeline we already have

Liliane Cavalcante - PhD Student
Federal University of Rio de Janeiro, Brazil

Interests - Data processing

Family: Anelloviridae
Species: Torque Teno Virus

Liliane Cavalcante - PhD Student
Federal University of Rio de Janeiro, Brazil
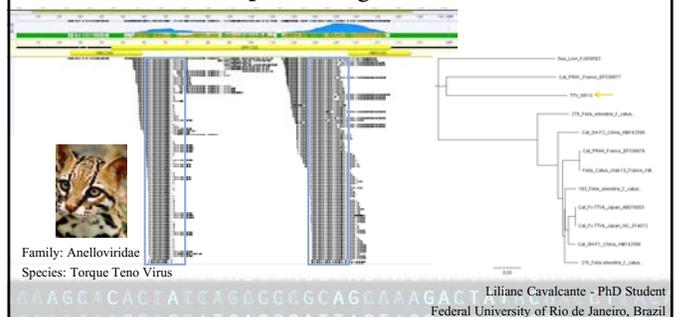
Christopher Cleveland
Guinea Worm Eradication Program
University of Georgia

Dracunculus medinensis & D. insignis

Is there an aquatic paratenic host?

Drinking contaminated water

Intermediate Host → Paratenic or transport host → Definitive Host
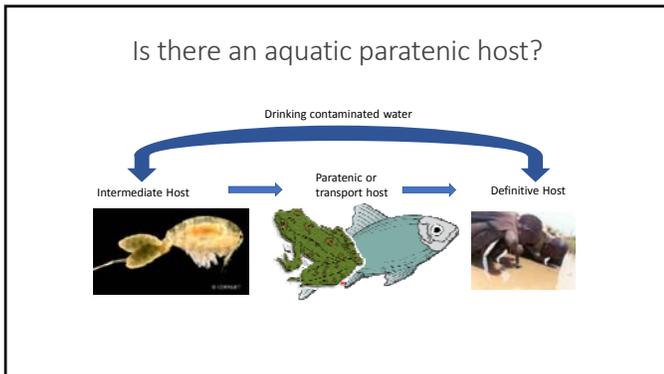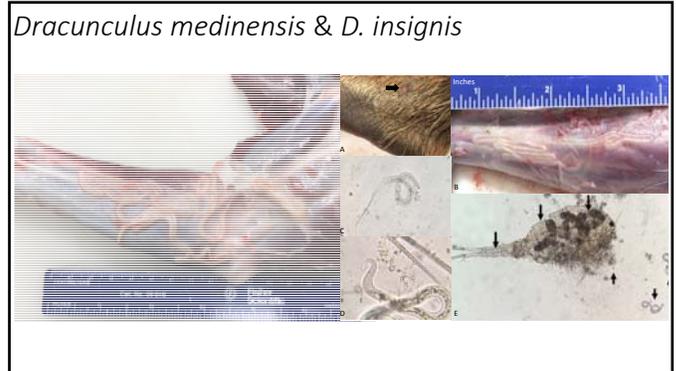
Guinea Worm Eradication Program
Chad, Africa
- Overall decrease in human cases
- Appearance and increase of dog infections since 2012.
- *Dracunculus medinensis* from humans and dogs genetically very closely related.
- Evidence of host switching among Dracunculids?
- Why are some Dracunculids host-specific vs. host generalists?
- How does this relate to the use of paratenic or transport hosts?

THE CARTER CENTER

Dr. Rick Gerhold
Wildlife Disease investigation and
classical and molecular parasitology
University of Tennessee

Training in wildlife disease investigations and classical and molecular parasitology

Investigating moose decline associated parasites

Estimated MN Moose Population

G.D. DelGiudice. 2014 Aerial Moose Survey Final Results. Report. Forest Wildlife Populations and Research Group, Minnesota Department of Natural Resources. St. Paul, MN.

Working on developing ELISA tests for both parasites

Detected emergence of *Elaeophora* both in moose from MN

UC Davis Veterinary Medicine

Interested in utilizing NGS tools for future research projects

Dr. Kristin Herrmann
Evology and evolution of helminth parasites
and their hosts
Tarleton State University

Background
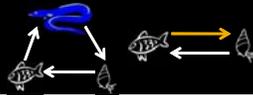
- B.S., University of Minnesota
- M.S., Minnesota State University Mankato
  - Thesis: Waterbird mortality associated with an invasive intermediate host of two trematodes
  - Advisor: Dr. Bob Sorensen
- Ph.D., University of Otago
  - Dissertation: Reproductive strategies of a trematode
  - Advisor: Prof. Robert Poulin
- Assistant Professor, Tarleton State University

Research Interests
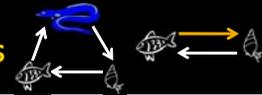
- Helminth parasites with complex life cycles
  - Ecology of host-parasite interactions
  - Communities and diversity
  - Cophylogeny of parasites and hosts
  - Cryptic species
  - Ecology and evolution of life history traits
- Other types of pathogens

Stephanie Hudon
Parasite Load and Telomere
Shortening in North American Raptors
Boise State University

BOISE STATE UNIVERSITY

- The gut microbiome of mule deer is hypothesized to adapt to enable them to feed on sagebrush in the winter which contains toxic chemicals

- Captive deer were fed a sage-free pellet diet and then shifted to a sagebrush diet and then returned to a pellet diet

- Fecal samples were collected for microbial analysis

- Expand the study to other herbivore-sagebrush systems such as white-tailed deer and pygmy rabbits

2

Parasite Load and Telomere Shortening in North American Raptors
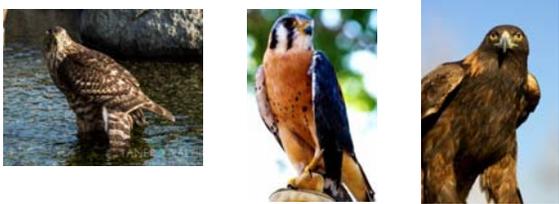
3

Avian Parasite Load and Telomere Senescence

Science. 2015 Jan 23;347(6220):436-8. doi: 10.1126/science.1261121.

Chronic infection. Hidden costs of infection: chronic malaria accelerates telomere degradation and senescence in wild birds.

Asghar M[1], Hasselquist D[2], Hansson B[2], Zehtindjiev P[4], Westerdahl H[2], Bensch S[2].

4

Lyndal Hulse
The Effect of Chlamydia on Koala Semen Quality
The University of Queensland

THE UNIVERSITY
OF QUEENSLAND
AUSTRALIA

Current status of South-East QLD Koalas

Conservation Status: Vulnerable [DEHP 2015]
Population Estimate: 79,264 (53% decline over 20 years)
(Adams-Hosking, McBride et al. 2016)

Threats to the species:
- Loss of habitat
- Trauma (car hits, dog attacks)
- Disease: Chlamydiosis

Estimated distribution of koalas in Queensland [DEHP 2015]

Chlamydia and Koalas

- Koalas are infected with two chlamydial species:

  C. pecorum and *C. pneumoniae*

- *C. pecorum* being the more pathogenic and widespread

Clinical syndromes associated with *C. pecorum* in the koala:

- Ocular infection
- Urinary tract infection
- Reproductive tract infections

<u>Objective</u>: Examine the effect of Chlamydia on koala semen quality with a view to developing methodologies to "clean-up" Chlamydia-infected semen for the purposes of artificial insemination and gamete recovery

<u>Project Aims</u>:
- Investigate the incidence of Chlamydia in semen of koalas in SEQ
- Detection of Chlamydia in semen and urogenital swabs correlated with clinical signs of chlamydiosis
- The effect of Chlamydia on sperm quality in koalas
- Transmission of Chlamydia using naturally-infected koala semen
- The effect of Chlamydia on spermatogenesis in the koala
- Antibiotic treatment of koalas and the effect on sperm quality: *in vivo* and *in vitro*

Future Directions

- Koala genome released on NCBI May 2017
- Begin investigating whether there is genetic association between koalas and Chlamydial Disease

AUSTRALIA ZOO
WILDLIFE HOSPITAL

DREAMWORLD WILDLIFE FOUNDATION

Queensland Government
Department of Environment and Heritage Protection

CURRUMBIN WILDLIFE HOSPITAL FOUNDATION

Melanie LaCava
Landscape Genomics of Wyoming Pronhorn and Mule Deer
University of Wyoming

CENTER FOR MOLECULAR DYNAMICS - NEPAL
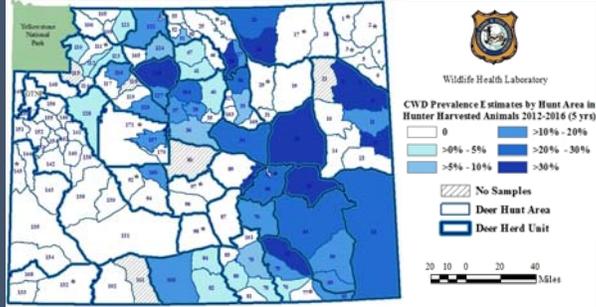
Parjwol Manandhar
Carnivor Disease Research in Nepal
University of Tampere

Research studies on wildlife disease at CMDN

- Bengal tiger (*Panthera tigris*) gut microbiome research

- **Carnivore disease project (Canine distemper virus)**

- Fecal viral metagenomics in tigers, snow leopards

- Tuberculosis in elephants/rhinos/boars/deer

- Avian influenza and Newcastle disease viruses in chicken and wild birds

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Carnivore disease project
Project site

Kathmandu valley

Bhaktapur

Carnivore disease project
Project site

Canine distemper virus (CDV)

Felidae|JungleCat

Felidae|LeopardCat

Felidae|Leopard

Bhaktapur District

Viverridae|Civet

Canidae|DomesticDog

&

Mustelidae|Marten

Canidae|Jackal

Carnivore disease project
Lab Procedures

RNA extraction and cDNA preparation

PCR to screen for Paramyxoviridae virus family

Gel electrophoresis to determine Positive results

Hemagglutinin (H) & Phosphoprotein (P) gene PCR followed by sequencing

Asia-1

America-2

Arctic

StrayDogsBhaktapur/Nepal

Asia-2

Europe

America-1
Vaccine

Phosphoprotein (P) gene
- Targeted fragment => 450 bp
- Trimmed fragment => 362 bp

Riley Mummah
Leptospira interrogans in the
California Channel Island Ecosystem
The University of California

Leptospira interrogans in the California Channel Island Ecosystem

o *Leptospira interrogans* serovar Pomona was first detected in California sea lions (CSL) in 1970 and and circulated continuously in sea lions from 1984-2013.

o *L. interrogans* serovar Pomona was discovered in 2009 on Santa Rosa Island in Channel Island foxes and spotted skunks.

o Subsequent serological studies have shown that it appears to have circulated in island foxes and other terrestrial mammals on Santa Rosa Island since at least the 1980s.

o The genetic profile (by VNTR analysis) of fox/skunk isolates from 2010 is nearly identical to isolates from CSL, raising questions about past transmission links.

Channel Island Fox

- Endemic to California
- Experienced a major population bottleneck in late 1990s
- Underwent captive breeding and all releases were complete by 2008

Leptospira interrogans

- Generalist pathogenic spirochete bacteria
- Infects mammals
- Classified by serovar
  - Does not map onto genetics
- 4.3 Mbp

A bit about me

- Lloyd-Smith lab at UCLA
- No background in genetics/genomics

- Using 48 isolates from 4 host species spanning 1988 to 2015
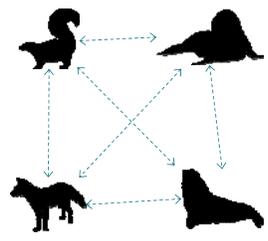
Nicole Nova
Phylogenetics and disease dynamics of canine distemper virus
(CDV) in Neartic carnivores
Stanford University

Canine distemper virus—wildlife threat · Stanford

Arctic fox (Alaska)

Amur tiger (Siberia)

Gilbert et al. unpublished

Amur local dogs
Arctic fox
Amur tiger

Canine distemper virus—wildlife threat · Stanford

Samples from Alaskan carnivores & mesocarnivores

Jort Vanderween

Canine distemper virus—wildlife threat · Stanford

Samples from Yellowstone carnivores & mesocarnivores

Jort Vanderween

Questions & Interests · Stanford

- What are the potential spillover pathways and reservoirs of CDV in Alaska?
- What is the rate of spread of CDV in wildlife over space, time, and across species in Alaska?
- How much do CDV strains vary between the outbreaks in Yellowstone wolves? Is each outbreak a result of an introduction of a new CDV strain?
- What is the rate of evolution of CDV in Yellowstone wolves? Are there specific sites undergoing positive selection?
- What is the likely origin of CDV in Yellowstone wolves?

Genomics (host & pathogen), population genetics,

phylogenetics, disease dynamics

Diana Prada
Pathogen Diversity and Host
Genetic Connectivity
Murdoch University

Microbats of the South West Botanical Province of Western Australia: Pathogen diversity and host genetic connectivity

Diana Prada, Mark O'Dea, Bethany Jackson & Peter Spencer, School of Veterinary and Life Sciences, Murdoch University, Perth, WA

Contact email: 32589004@student.murdoch.edu.au

Describe viruses in insectivorous bats within the region
- Do microbats of the SWBP carry viruses typical of this taxonomic group?

Associations by species, population or region
- Ecology of the host influences how pathogens move across populations
- Transmission routes

Genetic connectivity and diversity of four bat species
- Genetic diversity
- Level of connectivity amongst population
- Dispersal patterns

Bat species (SWBP)

Dr. Mathieu Pruvot
Wildlife Health Surveillance
Wildlife Conservation Society

LACANET

Building Wildlife Health Surveillance Networks

Wildlife disease outbreak investigation – PPRV in Mongolian Saiga

Drivers of zoonotic disease emergence

Land-use change

Wildlife trade

Land-use change in Cambodia

Intact forest

Maxomys sp.

Disturbed forest

Spillover?

Agricultural land

Mus spp

Spillover?

Genomics for:
- Species characterization
- Molecular epi
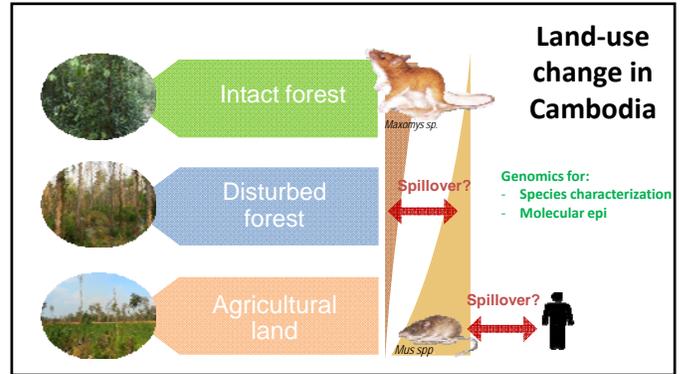
Wildlife Trade In Lao PDR

- Conservation impact
- Zoonotic disease risk

Genomics for:
- Pathogen characterization
- Pop genetic to identify trade routes

PPRV in Mongolia

Genomics for:
- Origin?
- How many spillover events?

Javier Sánchez Romano
Infectious Keratoconjunctivitis in semi-domesticated reindeer
The Arctic University of Norway

UiT
THE ARCTIC
UNIVERSITY
OF NORWAY

Infectious keratoconjunctivitis (IKC)

- Contagious ocular disease of ruminants worldwide.
  - Norway: occurs in sporadic outbreaks.

- Acute, rapid progression, clinical signs vary in severity.

- Painful, can impact vision or cause blindness – Reduced survival.

Clinical symptom score = 0
Clinical symptom score = 1
Clinical symptom score = 1
Clinical symptom score = 1

Clinical symptom score = 2
Clinical symptom score = 2
Clinical symptom score = 2
Clinical symptom score = 2

---

1. *Moraxella* spp. – Pathogenicity study

Is the isolate pathogenic?

Two virulence factors:

1. Type IV pili
2. Cytotoxin A

What about other isolates?

45 reindeer isolates from Norway, Sweden and Finland
16 cattle isolates from Denmark and Germany

36 isolates sequenced

Silvia Rondon
Intestinal parasites and Plasmodium spp. in non-human primates
Universidad de los Andes

Coverage of natural forests:
Less than **15%**

(Roncancio-Duque & Benavides Montaño, 2013)

2

Ateles hybridus
Brown spider monkey

Alouatta seniculus
Red howler monkey

Cebus versicolor
Capuchin monkey

Aotus griseimembra
Grey-legged night monkeys

Plasmodium falciparum

Plasmodium brasilianum

Plasmodium simium

Plasmodium malariae

Plasmodium vivax

4
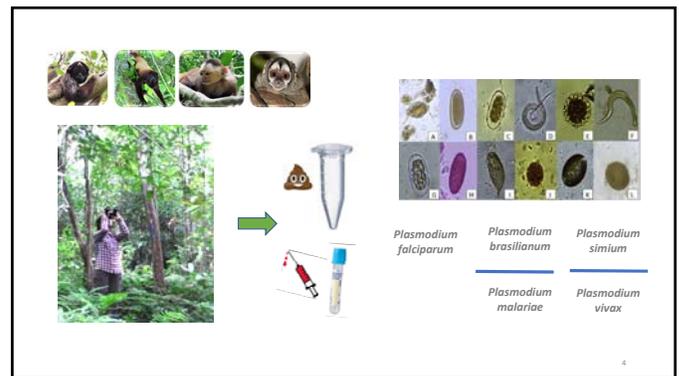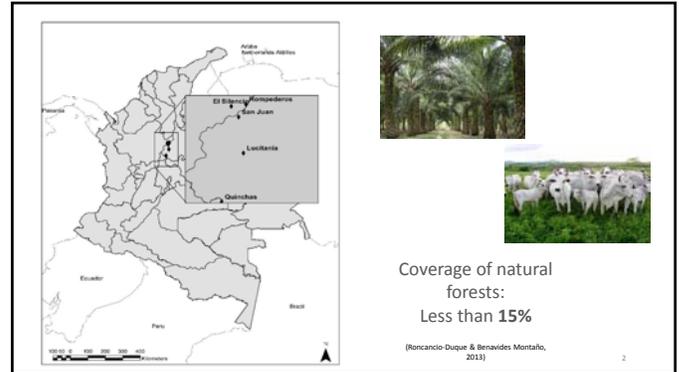
Dr. Todd Shurry
Using genomic tools to advance understanding of host-pathogen dynamics for B.abortus/M.bovis to advance conservation of wood bison and apply to other wildlife reservoirs
University of Saskatchewan

Title: Wildlife Health Specialist
Employer: Parks Canada Agency
Since: Feb. 2004
No. of veterinarians employed full-time by Parks Canada: 2
No. employees: ~ 4,000
Job Description: Constantly evolving – anything to do with wildlife health, veterinary medicine, One Health, zoonoses, wildlife disease, public health, ecohealth, animal care/welfare, epidemiology, wildlife policy......

Canada's National Parks System Plan

2

2015 PhD Thesis (U of Saskatchewan)
CHAPTER 5: SPATIAL AND MOLECULAR EPIDEMIOLOGY OF *MYCOBACTERIUM BOVIS* IN THE GREATER RIDING MOUNTAIN ECOSYSTEM

MIRU-VNTR and spoligotyping for *Mycobacterium bovis* isolates from elk, WT deer, cattle.

Recently WGSd 🙂

Surveillance for *Echinococcus canadensis* genotypes in Canadian ungulates

International Journal for Parasitology: Parasites and Wildlife

Molecular and morphological characterization of *Echinococcus* in cervids from North America

Main interest: Using genomic tools to advance understanding of host-pathogen dynamics for *B. abortus/M. bovis* (*B. anthracis* also!) to advance conservation of wood bison and apply to other wildlife reservoirs

As a result of recent tariffs imposed on our country by the Trump Administration......Beer and Maple Syrup will cost you more. HA!!

4

UNIVERSITY OF WYOMING
DEPARTMENT OF VETERINARY SCIENCES

Dr. Kerry Sondergoth
Determining "genotypes" of bacteria associated with pneumonia in Bighorn Sheep
University of Wyoming

Background

Bacterial Pathogens in BHS Pneumonia
- *Mannheimia haemolytica*
- *Bibersteinia trehalosi*
- *Pasteurella multocida*

Focus
- Isolates from 3 Wyoming herd units
- 2012-2018
- Diseased sheep samples
  - (Nebraska, Colorado, Wyoming)

	2012			2013			2014			2015			2016			2017			TOTAL		
	M	B	P	M	B	P	M	B	P	M	B	P	M	B	P	M	B	P	M	B	P
Absaroka	0	0	0	2	1	0	4	4	0	1	4	2	0	5	3	2	0	4	9	14	9
Jackson	5	0	0	0	0	0	4	0	0	0	0	0	2	0	0	2	0	1	13	0	1
Whiskey	3	3	0	0	0	0	2	2	0	1	1	0	0	0	2	1	1	1	7	7	3
																			29	21	13

Project Overview

Mass spectrometry "protein fingerprint" for bacterial ID

Tools
- Mass spectrometry
- Clin Pro Tools
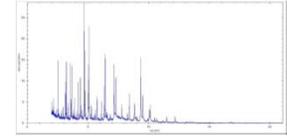- Whole genome sequencing

Characterize isolates
- Build bacterial ID database
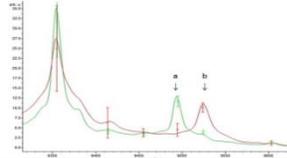- Compare isolates
  - Mass spec profiles vs sequencing

Microbiome analysis
- Tonsillar vs Nasal samples
- Sinus tumors

Mannheimia haemolytica "genotypes" in cattle

Expected Outputs and Goals

1. Bighorn Sheep Bacterial Respiratory Pathogen database
   - Differentiate species into genotypes
     - Requires WGS and Mass Spec data
   - Better diagnosis of bacterial isolates
   - *Learn how to handle and evaluate WGS for research AND diagnostics (workflow, choosing genes to generate comparisons, etc)*

2. Microbiome analysis
   - Tonsil vs Nasal swabs
     - Better inform wildlife biologists regarding sample collection and relocation
   - Predict sinus tumors based on microbiome?
   - Learn more about sinus tumor composition via tumor microbiome
   - *Become more competent with larger data sets and their manipulation/analysis for research*
   - *Learn how to effectively combine microbiome data (from individuals in herd) with overall health assessment data from the herd*

Dr. Nadine Vogt
Canadian Raptor Conservancy
Ontario Veterinary College

Wildlife Interests

- Canadian Raptor Conservancy
- DVM (2014)
  - Mortality in the Eastern Loggerhead Shrike recovery program
  - Minnesota Raptor Centre
- MSc Epidemiology (2017)
  - Thesis: epidemiology of *Campylobacter, Salmonella* and *E. coli* among Canada geese in Ontario
  - Volunteer at Toronto Wildlife Centre

Currently...

- PhD student in epidemiology at Ontario Veterinary College
- Advisors: Drs. David Pearl & Claire Jardine
- Thesis: Using whole genome sequencing to understand the ecology of *Salmonella* and antimicrobial resistance at the human, wildlife, environmental, and livestock interface in southern Ontario
- Research interests:
  - Zoonotic diseases
  - Evidence-based medicine
  - One Health research
  - Wildlife health

Goals for the workshop

- Background knowledge of genomics: limited
- Goals
  - Technical introduction to WGS methods
  - Strengthen analytical skills

Dr. Tiffany Wolf
Applied Infectious Disease
University of Minnesota

Applied Infectious Disease Research

- Moose health
  - *Parelaphostrongylus tenuis* epidemiology
- Primate health
  - At the interface with humans
- *Echinococcus* transmission
  - Domestic dog and human risk
- Tuberculosis transmission
  - Wildlife-livestock-human interface

Photo credit: Tiffany Wolf

Photo credit: Tiffany Wolf

Using Genomics to Understand Transmission

- Molecular epidemiology of *P. tenuis*
  - Landscape genetics
    - Genotyping of larvae from deer pellets
    - Examine transmission patterns across landscape
  - Metabarcoding of moose pellets
    - COX 1 marker gene
    - Exposure through the intermediate host

Adult Mortalities 2010-2015 (n=22)

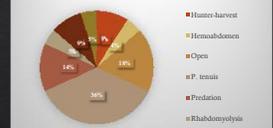- Hunter-harvest
- Hemoabdomen
- Open
- P. tenuis
- Predation
- Rhabdomyolysis

Photo credit: Seth Moore

Photo credit: Tiffany Wolf