

# PREDICTING DISEASE SPREAD IN GREATER YELLOWSTONE UNGULATES USING PARASITE DNA MARKERS

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## ♦ INTRODUCTION

Infectious diseases are a serious threat to the viability of wildlife populations worldwide, including those in national parks and other protected areas where agricultural operations, development, and recreation are degrading and fragmenting habitat and increasing the potential for interactions between wildlife, domestic animals, and humans. The spread of infectious diseases and parasites is of particular concern in the greater Yellowstone area, which supports world-renowned herds of ungulates that provide significant visitor enjoyment and benefits to local economies through guiding and sport hunting. The high diversity, density, and co-mingling rates of ungulates in this area could facilitate the rapid emergence and spread of infectious diseases such as brucellosis, chronic wasting disease, and Johne's disease, with escalating disease threats to livestock and people along the public/private land interface.

A critical need is information on disease and parasite transmission pathways within and among species and through the greater Yellowstone area to help develop feasible

strategies to minimize the adverse conservation,

economic, and social effects of diseases. We began addressing this need by identifying informative, polymorphic DNA markers from parasite propagules shed in ungulate feces that can be used to non-invasively identify, track, and map transmission routes across the greater Yellowstone area. Similar analyses of DNA markers from microparasites (e.g., viruses, bacteria) and macroparasites (e.g., helminths) in other areas have been used to determine the rates and routes of movement and disease spread by diverse host species such as cougars, salmon, and cattle (Blouin et al. 1995, Monis et al. 2005, Criscione et al. 2005, 2006; Biek et al. 2006).

The objectives of our study were to: 1) analyze the prevalence of parasites in elk and other ungulates inhabiting the northern and west-central portions of Yellowstone National Park and Grand Teton National Park in Wyoming; 2) identify polymorphic nuclear and mtDNA loci for macro- and micro-parasites; and 3) estimate genetic differentiation and transmission rates within and between host species and populations from the three sampling locations. This information will help natural resource managers throughout the greater Yellowstone area to

understand and map routes of spread of environmentally transmitted diseases such as brucellosis and chronic wasting disease, and predict the risks and routes of transmission.

#### ◆ METHODS

We collected 20 grams (e.g., 15-25 pellets) of fresh feces from elk, bighorn sheep, bison, pronghorn, and cattle from several geographic areas. Fecal samples were stored at 4° C for <1 month until parasites were isolated in the laboratory. A sub-sample of each fecal sample was also immediately placed in 95% ETOH and/or frozen for recovery of micro-parasites or direct PCR-based DNA analysis.

Helminth larvae were recovered from host fecal samples at the University of Montana using a modified Baermann method (Foryet 2001, Ezenwa 2004a). Parasite prevalence was estimated as the proportion of host individuals with the parasite (Ezenwa 2003, 2004b). DNA was isolated from individual larvae using commercial kits (e.g., DNAeasy tissue kits, Qiagen). Primers for PCR amplification and sequencing of mtDNA were available for *Haemonchus* and *Dictyocaulus* (Blouin 2002, Høglund et al. 2006). Primers for microsatellite genotyping and PCR-sequencing of many nuclear genes were also available (e.g., ITS-1, ITS-2; Blouin 2002, Wimmer et al. 2004).

To study a microparasite, *Brucella abortus*, we sampled 77 bison from slaughter houses (over ½ were seropositive for *Brucella*). We collaborated with other researchers and expect to obtain *Brucella* DNA isolates from an additional 50-100 bison, including animals migrating out of the northern and western boundaries of Yellowstone National Park. Also, we tested for *Yersinia enterocolitica* by culture from 30 bison feces from Grand Teton National Park. Isolation and culture of the bacteria was conducted in a commercial laboratory. DNA will be isolated from cultures (dead bacteria killed in 95% ETOH) or directly from fecal material using commercial kits (e.g., DNAeasy tissue kits, Qiagen). PCR primers for microsatellites and many nuclear genes are available for the bacteria species (e.g., Jourdan et al. 2000, Bricker and Ewaldt 2005, Sharma et al. 2006, Zheng et al. 2006).

DNA sequence analysis and estimation of polymorphism in each parasite species will be conducted using MEGA and Arlequin software (Luikart et al. 2001). Preliminary transmission rates will be estimated roughly as gene flow (i.e., migration) rates using population genetic distance statistics (e.g.,  $F_{ST}$ ) and models of population structure (e.g., island and stepping-stone models, using likelihood and Bayesian estimators; Beerli and Felsenstein 2001, Beerli 2006). Also, we will use assignment test approaches to directly identify recent transmissions without assumptions about population migration-mutation-drift equilibrium (e.g., Cornuet et al. 1999).

#### ◆ RESULTS

We sampled feces from bison, elk, bighorn, pronghorn, and/or cattle from Grand Teton National Park, the northern range of Yellowstone, along with 24 elk from Idaho on the Sand Creek wintering grounds. Prevalence of helminth parasites in each host species and each location are provided in Table 1.

We identified and optimized mitochondrial DNA primers for the ITS and NAD genes. We will soon PCR amplify and sequence ITS for species identification of approximately 8 nematodes from each host animal sampled to identify nematodes to species and to establish prevalence data. For one or two helminth species that are most prevalent, we will sequence NAD for approximately 30 worms per host species (elk, bison, pronghorn, and cattle) in each geographic location. This will allow for preliminary estimates of parasite population genetic structure (and transmission rates) within versus between host species and geographic locations.

We developed a quantitative real-time PCR test for *Brucella* DNA to help identify infected bison and elk. This test will be applied to feces, urine, blood and tissue. We also are genotyping 10 HOOF-print VNTR (variable number of tandem repeat or microsatellites) in collaboration with researchers at the National Animal Disease Center in Ames, Iowa (G. Luikart, manuscript in preparation). This will allow for estimation of transmission rates within and between elk and bison populations.

Location	No. fecal samples	Host species	<i>Protostrongylus</i>	<i>Dictyocaulus</i>	G.I. nematodes
Grand Teton NP	38	elk	0	0.22	0.1*
	32	bison	0.08	0.28	0.44
	19	bighorn sheep	1.0	0.05	1.0
	35	cattle (Pinto Ranch)	0.03	0	0.15
Yellowstone NP	20	elk	likely zero	0.56	0.75
	15	pronghorn	0.87 (genus uncertain)		0.8
	41	bison	0.13	0.1	0.76
Northern Wyoming (outside YNP and GTNP)	22	cattle	0.09 (genus not certain)		0.53
	13	pronghorn	0.92 (genus uncertain)		0.91
Bench/Jewett Feed Grounds	20	elk	likely zero	0.58	0.08

Table 1. Prevalence of helminth parasites in ungulate host species from the greater Yellowstone area.

\* These elk samples were rather old when analyzed, so prevalence estimates are likely biased low.

No *Yersinia* positives were identified in 30 bison feces from Grand Teton National Park. We currently are testing 120 more fecal samples from bison on their summer range and those consigned to slaughter from Yellowstone National Park, as well as samples from bison in Grand Teton National Park during winter. We will be testing these fecal samples, plus many fecal samples from elk, for *Yersinia enterocolitica* (strain 0:9) by PCR directly from feces and lymph node DNA isolates. Once PCR or culture identifies *Yersinia*, we intend to sequence a few gene fragments to identify polymorphisms for studying transmission, depending on funding availability.

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