Anne Acton, DVM
NC STATE UNIVERSITY COLLEGE OF VETERINARY MEDICINE
Raleigh, North Carolina 27606
(919) 513-6653 office
aeacton@ncsu.edu

2004 EPA STAR Graduate Fellowship Conference *Next Generation Scientists—Next Opportunities*

Noninvasive Wildlife Health Monitoring Using Molecular Detection of Fecal Pathogens

Overview

Disease, diet and distribution play important roles in the health of free-ranging wildlife populations. The ability to assess disease risks without locating or handling individual animals would overcome major logistical problems in wildlife health management. Our focus is on the **use of field-collected feces as a noninvasive sampling strategy to detect key pathogens and associated prey that can impact endangered species reintroduction programs.**

Research Approach

Fecal PCR and rt-PCR assays are being developed to detect parvoviruses (including feline panleukopenia, canine parvoviruses (CPV), and raccoon parvovirus) and canine distemper virus (CDV) which have high impact potential on carnivore mortality. Also in development are PCR assays targeting selected prey mtDNA in carnivore feces. Of interest are: **1) lower detection limits for each assay; 2) duration of fecal shedding following exposure to virus or prey; and 3) effects of fecal degradation on target recovery.**

Assay sensitivity and specificity are determined from control feces spiked with known concentrations of virus or prey tissue. Feeding and vaccination trials will assess assay reliability. Following laboratory validation, field application of the fecal viral assays will be tested in conjunction with serology of 5 sympatric carnivore species[a] from eastern North Carolina where red wolves have been reintroduced. Sample collection over multiple seasons is in collaboration with the USFWS Red Wolf Program, the NC Wildlife Resources Commission and local trappers.

Acknowledgements: EPA STAR and Morris Animal Foundation grants support this research. Pilot project funding was kindly provided by the USFWS Red Wolf Program.

Preliminary Results

Assay Specificity

Virus Source	Primers PV[b]	Primers CDV[c]
CPV[d] Type 2b	563 bp	-
CPV[e] Type 2	562 bp	-
CDV[e] Synder Hill	-	286 bp
CDV[f] Ondestepoort	-	287 bp

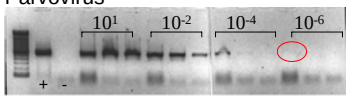
Prey Primers[g]	Product Size
WT deer	265 bp
raccoon	793 bp
red wolf	110 bp

Amplicons were sequenced and BLASTed in GenBank to verify intended targets.

Assay Sensitivity

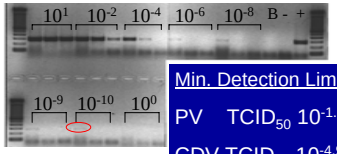
Virus detection from feces spiked with serial dilutions of vaccine[d,f]

Parvovirus



Assay sensitivity was stable after 3 days @ 22ºC for CPV only.

Canine distemper virus



Min. Detection Limit
PV $TCID_{50}$ $10^{-1.6}$
CDV $TCID_{50}$ $10^{-4.9}$

Prey Deer assay detected as little as 0.3mg tissue in 1g feces.

Storage Effects

- **PCR(+)s obtained from vaccine-spiked feces held at room temp for ≤ 7d.** Initial concentrations were approx. $TCID_{50}$ $10^{3.5}$ for CPV and $TCID_{50}$ $10^{3.6}$ for CDV per 1g feces.
- **Feces from deer food trial remain PCR(+) at 90d in room temp, 4ºC, guanidine and EtOH.** No false positives resulted from prolonged storage.

What's Next?

In the Lab:

- Conduct vaccine trials using modified-live products in both previously exposed and naïve animals to model the fecal viral shedding in natural infections
- Expand study of storage conditions and media effects on virus recovery from feces
- Investigate additional targets to improve detection of virus and prey in degraded samples

Field Applications:

- Perform comparative serology and fecal viral assays on 2003-2005 samples to estimate disease exposure risks in study area
- Employ GIS technology to discern distribution patterns of assay results based on selected environmental attributes

Expected Impact

Combining laboratory validated fecal assays for pathogens and geographic information systems (GIS) technology can provide more useful data for population health management decision-making than traditional serologic surveys. Important advantages include discrimination of active infections with the potential for spread and a better understanding of the spatial components of disease maintenance in a region.

Graduate Committee: M Stoskopf (Clinical Sciences), J Guy (Population Health & Pathobiology), R Powell (Zoology), G Hess (Forestry)

Notes:

a. Black bear (*Ursus americanus*), bobcat (*Felis rufus*), coyote (*Canis latrans*), gray fox (*Urocyon cinereoargenteus*), raccoon (*Procyon lotor*)
b. VP2 region; PV forward: 5'ACACATACATGGCAAACAAATAGA3'; PV reverse: 5'ACTGGTGGTACATTATTTAATGCAG3' **[J Gen Virol 81: 345-350]**
c. NP gene; CDV forward:5'ACAGGATTGCTGAGGACCTAT3'; CDV reverse: 5'CAAGATAACCATGTACGGTGC3' **[J Clin Microbio 37(11): 3634-3643]**
d. Galaxy®PV modified-live canine parvovirus (Schering-Plough Animal Health, Union, NJ USA 07083)
e. Vanguard®5 modified-live canine distemper, adenovirus Type2, parainfluenza, parvovirus (Pfizer Animal Health, Exton, PA USA 19341)
f. Galaxy®D modified-live canine distemper virus (Schering-Plough Animal Health, Union, NJ USA 07083)
g. Species-specific primers targeting varied sized products in the cytochrome b gene region of mtDNA

This fellow is sponsored by EPA's STAR or Greater Research Opportunities (GRO) Program.