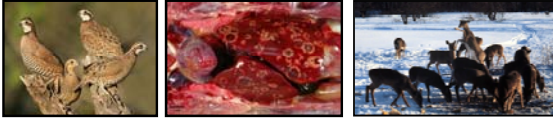


Using disease research in wildlife conservation



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Examples of disease research in wildlife



Examination of diseases in captive northern bobwhites



Northern bobwhites are an important game species

- Popular game species
 - 2.9 million hunters spent \$1.3 billion in 2006
- Regional population decline
 - Habitat loss



Northern bobwhite trends, 1966-1996



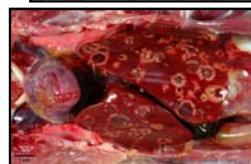
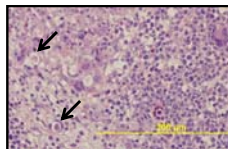
Captive rearing of quail for hunting is big business

- Birds raised in captive facilities → released as adults
 - 20-30 million/yr in U.S.
- High bird density results in efficient pathogen transmission
 - Blackhead (*Histomonas*)
 - Ulcerative colitis (*Clostridium colini*)
 - Coccidiosis (*Eimeria* spp.)



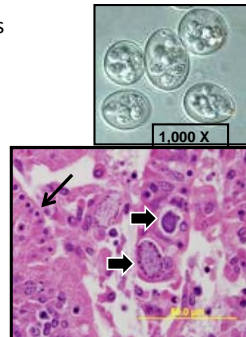
Histomonosis is important disease in domestic and wild turkeys

- Re-emerging disease in commercial turkeys
 - Mortality events >85% of flock reported
- Second most frequently diagnosed disease in wild turkeys from S. E. US



Coccidiosis is very common in captive raised animals

- Microscopic protozoal parasites
- Environmentally resistant stage
- Intestinal cells infected in host
 - Very prolific replication
 - 1 ingested oocyst can produce > 50,000 oocysts/ bird
- Can cause large mortality and morbidity events



Our research questions

- 1) What is the prevalence and geographical distribution of coccidia spp. from captive bobwhite farms?
- 2) Are there drug resistant strains of coccidia from farms using anticoccidials to treat and prevent coccidiosis?

Captive bobwhite litter samples originated from 12 states



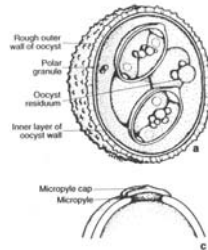
- 31 samples collected from 12 states
 - Flock age: 2 weeks → adult
- Propagated in bobwhites → Xenodiagnosis

Classical identification of game bird oocysts was problematic due to similar morphology

- Three previously described *Eimeria* spp. in bobwhites

- Classical identification

- Morphology of oocysts
- Gross and histological lesions



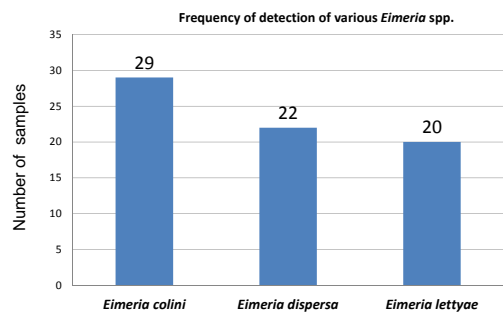
Species specific primers developed and tested for specificity

- Use genus wide *Eimeria* spp. primers BSEF and BSER – Amplified (ITS-1) region of rRNA
- Construct primers to nucleotide sequences conserved among *Eimeria* spp. within group, but different than other groups
- Forward and reverse primers constructed

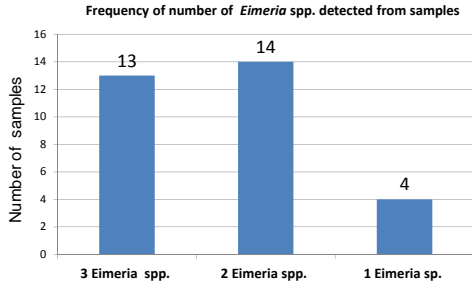
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NOBO 02 C1 AATTATAAATTGTGTTGTACTGTGCACACCCATGGAGCAAACCGTA
NOBO 02 C5 ...TAA...T...CG...AG...A...T...CG...
NOBO 03 C3 ..GCCATTCAACGTTTCACG.....G.....A.....T...G.....
NOBO 05 C3 ..GCCATTCAACGTTTCACG.....G.....A.....T...G.....
NOBO 06 C1 ..GCCATTCAACGTTTCACG.....G.....A.....T...G.....
NOBO 02 C5 ..GCCATTCAACGTTTCACG.....G.....A.....T...G.....
    
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All farms contained at least one species of coccidia



Majority of farms had at least two *Eimeria* spp. present



Discussion: survey results

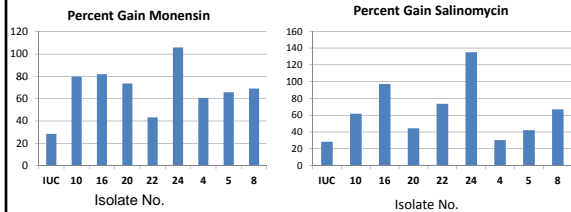
- First known survey of captive bobwhite farms
 - 100% of farms contained coccidia
 - 27 (87.1%) samples had at least two species
 - No associations with geographical location or flock age
- Research needed to understand the effects on wildlife

Identification of anticoccidial resistant *Eimeria* spp. in northern bobwhites

Examination of resistance in field isolates

- Anticoccidial trials performed similar to those of domestic poultry
 - 1 pen 10 birds each
 - 6 to 10 field isolates used per compound
 - Monensin (90 ppm)
 - Salinomycin (55 ppm)
- Used percent weight gain of birds as index of drug efficacy
 - Compared to uninfected controls

Resistance to monensin and salinomycin observed in multiple isolates



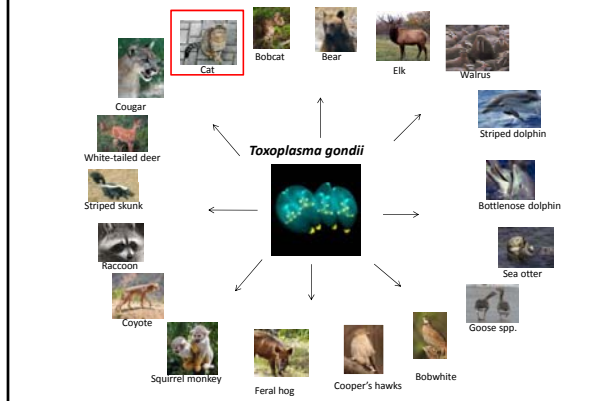
Summary of anticoccidial study

- At least half of the tested isolates were resistant to at least one of the anticoccidial compounds
- Demonstrates that captive quail farming is selecting for resistant strains of bobwhite coccidia
 - Potential contamination of environment with resistant strains

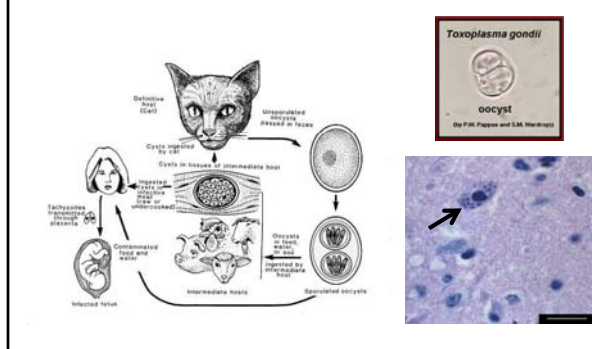
How does this research relate to conservation

- Propagation of captive bobwhites are frequently seen as a “fix” for declining wild quail populations
 - Leads to less emphasis on habitat conservation
 - Dangerous road to head down
- Our research demonstrates the significant disease ramifications of captive quail propagation
 - Use information in our efforts to promote habitat conservation for quail restoration

Transmission of *Toxoplasma gondii* in wildlife



***Toxoplasma gondii*: felids are the only definitive hosts**



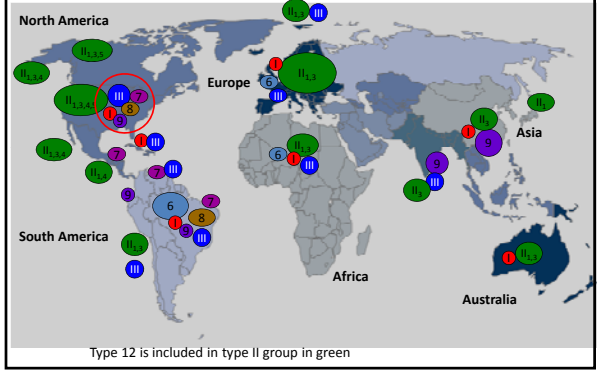
Humans are most often infected by oocysts

- *T. gondii* sporozoite specific protein allows for serological distinction between oocyst and tissue cyst.
- 63.2% (N=103) humans were positive for sporozoite specific protein.
 - Points to felids as major source of infection



Hill et al., 2011. Journal of Parasitology 97: 328-337

Genotypes of *T. gondii* vary depending on geographical distribution with type I & III being more virulent



Why are we seeing various genotypes in eastern US?

1. Previously undetected genotypes
2. Importation of exotic animals from South America and other countries into the US
3. Increase in number of definitive hosts where sexual replication of parasite occurs



Transmission of *Toxoplasma gondii* in wildlife in eastern US

Funded by Microbiology across Campuses Educational and Research Venture (M-CERV)

- Designed to assist collaboration amongst microbiologists from the UT campuses in Knoxville

Collaborative research among:

- Center for Wildlife Health
- College of Veterinary Medicine
- Microbiology Department



Experimental design

- Collect fresh (non-frozen) heart muscle from wild mammals and birds from eastern US
 - Hunter-killed, road-killed, nuisance killed, trapper collected, etc.
- Test serum for antibodies to *T. gondii*
 - Using modified agglutination test at veterinary school
- Seropositive heart samples are used to propagate *Toxoplasma* parasites for genotyping
- Data used as model of parasite transmission

Results to date have been very encouraging

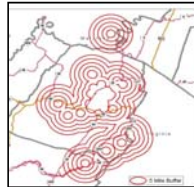
- Collected and tested 130 samples for *T. gondii* antibodies
 - White-tailed deer, mink, raccoon, Virginia opossum, coyote, gray fox, elk, American crow, woodchuck, gray squirrel, black bear,
- 47 (36.4%) seropositive animals were detected.
 - 37 deer, 3 raccoons, 2 elk, 3 coyotes, 1 mink, 1 opossum
- *Toxoplasma* parasites have been successfully propagated from 1 mink and 9 deer.

Preliminary genotyping data

ID	Species	SAG1	5-3'SAG2	BTUB
17	Mink	II	II	II
40	White-tailed deer	u-1	II	II
43	White-tailed deer	u-1	II	II
60	White-tailed deer	u-1	II	II
78	White-tailed deer	u-1	II	II
88	White-tailed deer	I	II	II
98	White-tailed deer	II	I	I
99a	White-tailed deer	u-1	II	II
110	White-tailed deer	I	I	III
113	White-tailed deer	u-1	II	II

Future plans --use preliminary data for an NSF-EEID proposal

- Focus on areas where there are known feral cat colonies and exotic importations
- Perform targeted surveillance surrounding these areas and those with unique genotypes
- Identify the major vertebrates associated with *T. gondii* transmission
- Create predictive model for introduction and maintenance of novel genotypes



How is this important for conservation?

- Identifies wildlife disease surveillance as a predictor for human pathogens
 - Allows us to tap into existing wildlife projects and further demonstrate the usefulness for wildlife research
- Further demonstrates the negative impacts of non-native animal populations
 - Exotic animal importation
 - Free roaming cats and TNR

Questions??
