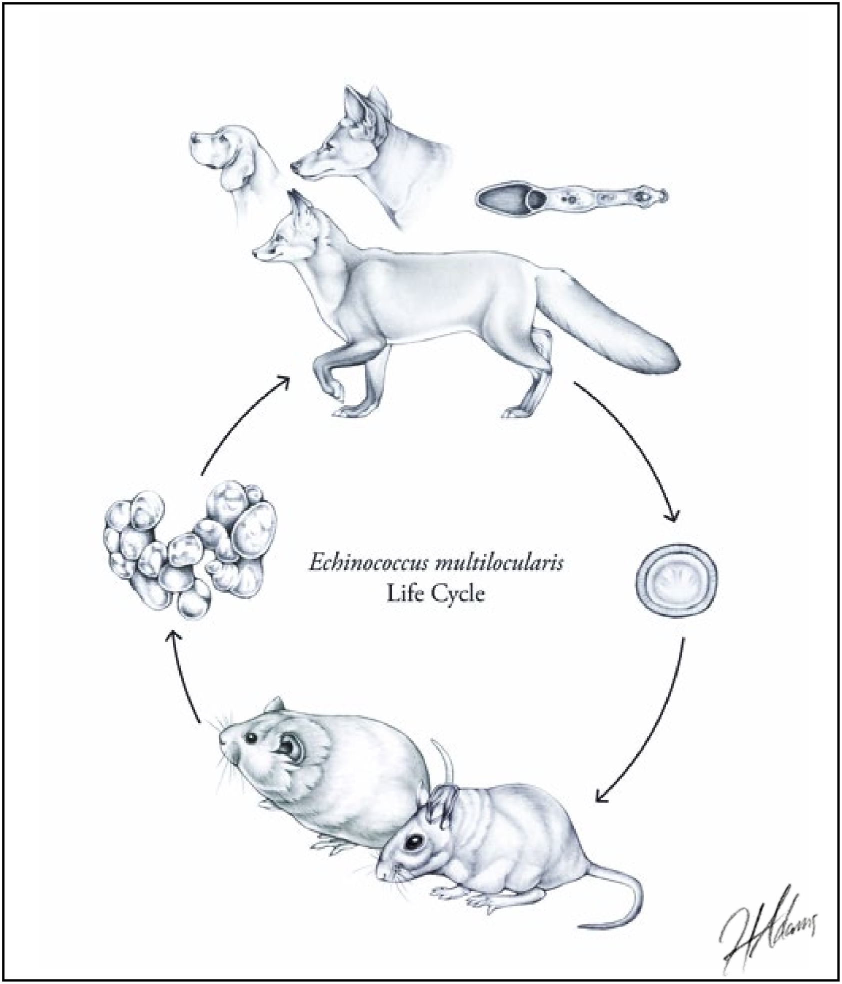
SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY
2020 ANNUAL REPORT

July 1, 2019 – June 30, 2020



College of Veterinary Medicine
University of Georgia
Athens, Georgia

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY

2020 ANNUAL REPORT

July 1, 2019 - June 30, 2020

College of Veterinary Medicine
The University of Georgia
Athens, Georgia

The Southeastern Cooperative Wildlife Disease Study is the first regional research and service organization established in the United States for the specific purpose of investigating diseases of wildlife and providing assistance to wildlife management agencies. The project is supported by the University of Georgia and the Southeastern Association of Fish and Wildlife Agencies. Funds for the cooperative effort are administered and research is coordinated under the Federal Aid in Wildlife Restoration Act (50 Stat. 917) and through cooperative agreements with the U.S. Geological Survey and the U.S. Fish and Wildlife Service, U.S. Department of the Interior, and with the Animal and Plant Health Inspection Service, U.S. Department of Agriculture. Participating fish and wildlife agencies include those of: Alabama, Arkansas, Florida, Georgia, Kansas, Kentucky, Louisiana, Maryland, Mississippi, Missouri, Nebraska, North Carolina, Oklahoma, Pennsylvania, South Carolina, Tennessee, Virginia, and West Virginia.

TABLE OF CONTENTS

SCWDS STEERING COMMITTEE

Paul Johansen, Chair	West Virginia Division of Natural Resources
Chuck Sykes, Vice Chair	Alabama Department of Conservation and Natural Resources
Michael Piccirilli, Secretary	U.S. Fish and Wildlife Service
Lisa Nolan, Dean	College of Veterinary Medicine, The University of Georgia
David Stallknecht, Director	Southeastern Cooperative Wildlife Disease Study
Cory Gray	Arkansas Game and Fish Commission
Mark Cunningham	Florida Fish and Wildlife Conservation Commission
Rusty Garrison	Georgia Department of Natural Resources
Jake George*	Kansas Department of Wildlife, Parks, and Tourism
Karen Waldrop	Kentucky Department of Fish and Wildlife Resources
Kenny Ribbeck	Louisiana Department of Wildlife and Fisheries
Karina Stonesifer*	Maryland Department of Natural Resources
Anthony Ballard	Mississippi Department of Wildlife, Fisheries, and Parks
Sherri Russell	Missouri Department of Conservation
Tim McCoy*	Nebraska Game and Parks Commission
Cameron Ingram	North Carolina Wildlife Resources Commission
Jerry Shaw	Oklahoma Department of Wildlife Conservation
Matthew Schnupp	Pennsylvania Game Commission
Charles Ruth	South Carolina Department of Natural Resources
Chuck Yoest	Tennessee Wildlife Resources Agency
Gray Anderson	Virginia Department of Wildlife Resources
Samantha Gibbs	U.S. Fish and Wildlife Service, Wildlife Health Office
Camille Hopkins	U.S. Geological Survey, Ecosystems Mission Area
Jonathan Gassett	Wildlife Management Institute

* Associate Member

SCWDS OBJECTIVES

- To detect causes of morbidity and mortality in wildlife.
- To define the impact of diseases and parasites on wildlife populations.
- To delineate disease interrelationships between wildlife and domestic livestock and poultry.
- To determine the role of wildlife in the epidemiology of human diseases.

Our basic philosophy is to work for the benefit of wildlife resources and animal health. We believe that by adhering to this philosophy SCWDS will be serving the current and future needs of our sponsors and providing benefits to each cooperator far beyond what could be purchased with any member's individual contribution.

SCWDS PRIORITIES FOR FY2020

The Steering Committee assists SCWDS in identifying which activities should have priority on an annual basis. This year's priorities were set as follows:

- Respond to requests for immediate diagnostic, consultative, or field assistance in regard to wildlife health matters as problems arise. In addition to state and federal conservation and animal health agencies that support SCWDS, services will be provided to state animal health authorities, public health officials, wildlife researchers, and others when it is in the interests of wildlife conservation and when SCWDS resources are available.
- Prepare informational materials on salient wildlife diseases to increase understanding of disease biology and to help wildlife, animal health, and public health agencies communicate with the public and the news media.
- Monitor major selected wildlife diseases to determine their impact on populations and to search for epidemiologic features that could be used by wildlife managers to predict and avoid problems.
- Provide deer herd health evaluation services to state fish and wildlife management agencies within the southeastern region where health problems are suspected or data are needed to make controversial deer management decisions. Deer herd health evaluation services will be provided to other organizations or individuals when SCWDS resources are available and with the concurrence of the state fish and wildlife agency.
- Conduct or assist with the development and field testing of methods for disease prevention and control in wildlife populations.

FACULTY AND STAFF

SCWDS Faculty

David E. Stallknecht, PhD, SCWDS Director and Professor
Christopher A. Cleveland, PhD, Assistant Professor
Sonia M. Hernandez, DVM, PhD, DACZM, Professor
Daniel G. Mead, MPH, PhD, Professor
Nicole M. Nemeth, DVM, PhD, DACVP, Associate Professor
Rebecca Poulson, PhD, Assistant Research Scientist
Mark G. Ruder, DVM, PhD, Assistant Professor
Michael J. Yabsley, PhD, Professor
Joseph L. Corn, PhD, Professor Emeritus
John R. Fischer, DVM, PhD, Professor Emeritus

SCWDS Staff

Erin Box, BS, Research Technician III
Deborah Carter, BS, Research Professional I
Jeanenne P. Brewton, Administrative Associate II
Emily Doub, BS, Research Technician III
Brian Dugovich, DVM, PhD, Post-Doctoral Research Associate
Patricia A. Flaherty, BA, Administrative Specialist I
Alinde Fojtik, BS, Research Professional I
Kayla Buck Garrett, MS, Research Professional I
Jenna Gettings, DVM, MPH, SCWDS Post-Doctoral Research Associate
Kayla Guinn Adcock, MS, Research Technician III
Jennifer T. Smith, Laboratory Technician III
Natalie Stilwell, DVM, PhD, Post-Doctoral Research Associate
Michael Tanner, MS, Research Technician III
Stacey L. Vigil, MS, Research Professional III
Seth White, BS, Research Technician III
Sarah Williamson, BS, Business Manager I
Michelle Willis, MPH, Research Professional I
John C. Wlodkowski, BS, Research Professional I

SCWDS Graduate Students

Henry Adams, BS, Graduate Research Assistant
Andrea Ayala, MS, Graduate Research Assistant
Katherine Christie, Graduate Research Assistant
Jo Anne Crum Bradley, MS, Graduate Research Assistant
Katherine Franc, BS, Vet Med Graduate Research Assistant
Raquel Francisco, DVM, Graduate Research Assistant
Ryan Grunert, BS, Graduate Research Assistant
Melanie R. Kunkel, DVM, MPH, Vet Med Graduate Assistant
Rebecca Radisic, DVM, Wildlife Pathology Resident
Jorge Rojas, DVM, Graduate Research Assistant
Julia Silva-Seixas, BS, Graduate Research Assistant
Alec Thompson, MS, Vet Med Graduate Research Assistant
Alisia Weyna, DVM, Wildlife Pathology Resident
Brianna Williams, MS, Graduate Research Assistant
Seth Wyckoff, BS, Graduate Research Assistant

SIGNIFICANT DEVELOPMENTS OF THE YEAR

Wildlife Mortality Investigations

The SCWDS Diagnostic Service received 997 accessions from July 1, 2019 through June 30, 2020, including carcasses or samples from 1,356 animals. These included 377 specimens from 52 avian species, 918 samples from 35 mammalian species, 47 samples from 11 reptilian species, and 14 samples from 3 amphibian species.

Species Represented Among SCWDS Diagnostic Accessions
July 1, 2019 - June 30, 2020

Birds	
American Crow	11
American White Ibis	13
American Wigeon	1
Bald Eagle	50
Black Skimmer	2
Black Vulture	4
Black-billed Magpie	2
Black-throated Blue Warbler	1
Brown Pelican	5
Brown-headed Cowbird	3
Burrowing Owl	5
Canada Goose	5
Cedar Waxwing	2
Common Eider	3
Common Grackle	23
Common Ground Dove	1
Common Starling	5
Cooper's Hawk	1
Double-crested Cormorant	5
Finch (unknown species)	2
Gadwall	1
Golden Eagle	12
Great Blue Heron	1
Great Egret	1
Green Heron	4
House Finch	1
House Sparrow	64
Laughing Gull	7
Mallard	1
Masked Bobwhite Quail	5
Mississippi Kite	1
Mourning Dove	5
Muscovy Duck	6
Northern Bobwhite Quail	9
Northern Cardinal	1
Osprey	1
Palm Warbler	2
Passerine (unknown species)	2
Puerto Rican Amazon	15
Purple Martin	5
Red-eyed Vireo	1
Red-tailed Hawk	3
Ruffed Grouse	1
Sandhill Crane	7
Snowy Egret	1
Sparrow (unknown species)	1
Tree Swallow	10
Turkey Vulture	3
Wild Turkey	59
Wood Duck	1
Wood Stork	1
Yellow-crowned Night Heron	1
Total	**377**
Mammal	
American Beaver	3
American Black Bear	37
American Mink	1
Big Brown Bat	24
Brazilian Free-tailed Bat	27
Bobcat	14
Eastern Cottontail	6
Eastern Gray Squirrel	10
Eastern Red Bat	3
Eastern Spotted Skunk	7
Elk	57
Evening Bat	9
Florida Bonneted Bat	2
Florida Panther	1
Fox Squirrel	5
Gray Fox	10
Groundhog	1
Indiana Bat	1
Least Weasel	1
Little Brown Bat	3
Mountain Lion	3
Mule Deer	115
Northern Flying Squirrel	3
Pronghorn	6
Raccoon	30
Red Fox	8
Sika Deer	1
Silver-haired Bat	1
Snowshoe Hare	1
Southeastern Myotis	1
Southern Flying Squirrel	21
Striped Skunk	9
Virginia Opossum	4
Western Small-footed Bat	1
White-tailed Deer	492
Total	**918**
Reptiles	
American Alligator	5
Bog Turtle	11
Eastern Box Turtle	21
Eastern Indigo Snake	1
Eastern Ratsnake	1
Florida Box Turtle	1
Gopher Tortoise	1
Green Iguana	1
Painted Turtle	3
Red-eared Slider	1
Scarlet Kingsnake	1
Total	**47**
Amphibians	
Gopher Frog	5
Green Frog	7
Spotted Salamander	2
Total	**14**
Cumulative	**1356**

Noteworthy cases and summaries of select general and specific etiologies includes the following:

Infectious Diagnoses

Rabies was diagnosed in three striped skunks (two from Kansas and one from Nebraska), two raccoons (one from North Carolina and one from West Virginia), and two Mexican free-tailed bats and a white-tailed deer from Georgia.

Distemper (due to canine morbillivirus) was diagnosed in 23 raccoons (six from Kentucky, four from Arkansas, four from North Carolina, three from Kansas, three from Louisiana, two from Pennsylvania, and one from Georgia), seven gray fox (five from North Carolina and two from Georgia), five eastern spotted skunk from North Carolina, three striped skunk from Kansas and one black bear from Pennsylvania. Fourteen of these animals had comorbidities (e.g., concurrent lungworm or bacterial infections).

Fourteen wild turkeys were diagnosed with avian pox, five from Pennsylvania, three from Kansas, three from Tennessee, two from Kentucky and one from South Carolina. Lymphoproliferative disease virus (LPDV) was detected in 11 of these turkeys with pox, reticuloendotheliosis virus (REV) in three, and both viruses were detected in nine wild turkeys with avian pox. Overall, 30 wild turkeys tested positive for LPDV genetic material (21 from Pennsylvania, two each from Kansas, Kentucky, and Tennessee, and one each from Florida, North Carolina and South Carolina), most (21; 70%) of which also tested positive for REV genetic material. Three LPDV-positive turkeys had lesions consistent with lymphoproliferative disease.

Less commonly diagnosed viral infections included West Nile virus in a bald eagle from Florida, duck viral enteritis virus (i.e., anatid herpesvirus-1) in three Muscovy ducks (two from South Carolina and one from Florida), circovirus in a laughing gull from Florida, and parvovirus in a raccoon from Virginia.

Pathogen detections in herpetofauna included ranavirus detection in three green frogs from West Virginia, and eight eastern box turtles from South Carolina (frog virus-3). Turtle herpesvirus was detected in seven eastern box turtles from South Carolina, and *Mycoplasma* spp. (turtle type) was detected in 16 eastern box turtles from South Carolina. *Batrachochytrium dendrobatidis* was detected in one green frog from West Virginia. *Ophidiomyces ophiodiicola*, the cause of snake fungal disease, was detected in two snakes (details are below in the Snake fungal disease section).

Bacterial infections included *Francisella tularensis* (causative agent of tularemia) in three eastern cottontails, two from Kansas and one from Kentucky. *Salmonella* sp. associated with multi-systemic disease was detected in three brown-headed cowbirds, two from Louisiana (serotype: Typhimurium) and one from Florida (*Salmonella enterica* group B), as well as a great egret from Maryland (*Salmonella enterica* Group B). Thirty-one cervids had bacterial infections that ranged from brain abscesses (one mule deer from Idaho and one white-tailed deer from Georgia) to pneumonia and more generalized infections (24 white-tailed deer; seven from West Virginia, four each from Georgia and Louisiana, three from South Carolina, two from North Carolina, and one each from Arkansas, Mississippi, Tennessee, and Wisconsin).

Fourteen white-tailed deer had pneumonia attributed to infection with fungi, fungus-like organism (water mold), or algae. *Fusarium* sp. fungus was cultured from one of these. These deer were from Georgia (four), Pennsylvania (three) and Arkansas, Florida, Maryland, Nebraska, North Carolina, Virginia and West Virginia (one each). All but one of these deer was euthanized or died from September to December.

Protozoan infections included *Trichomonas gallinae* in three mourning doves (two from Georgia and one from Arkansas), one common ground dove from Florida, and one house finch from West Virginia. A wild turkey from Pennsylvania had gastrointestinal disease with detection of *Tritrichomonas* sp. An American beaver from North Carolina had brain inflammation associated with *Toxoplasma gondii* infection; this protozoan also was detected in tissues from a wild turkey in Pennsylvania. *Sarcocystis* sp. infections were diagnosed in a bald eagle with brain inflammation from Virginia and a burrowing owl from Florida.

Sixty-five cervids were diagnosed with aberrant meningeal worm (*Parelaphostrongylus tenuis*; suspect, presumed or confirmed based on lesions and presence of associated nematodes). These included 26 elk (22 from Kentucky, 2 from West Virginia, and one each from Kansas and Nebraska) and 39 mule deer from Nebraska.

Skin disease included various ectoparasites (mites) and bacteria; sarcoptic mange (caused by *Sarcoptes scabiei* mites) in black bears was most commonly diagnosed and included 31 bears, all from West Virginia. In addition, three red foxes were diagnosed with sarcoptic mange (one each from Kansas, Nebraska, and Virginia). Ten cervids were diagnosed with dermatophilosis (*Dermatophilus congolensis* [bacterial] infection of the skin); this included five white-tailed deer (three from West Virginia, one from Louisiana and one from South Carolina), two mule deer (one from Kansas and one from Nebraska), one elk from Kentucky, and one pronghorn from Kansas. Demodectic mange (caused by *Demodex* sp. hair follicle mites) was diagnosed in two white-tailed deer, one from Georgia and one from Louisiana, and in an Indiana bat from West Virginia.

Chronic wasting disease was diagnosed in seven cervids from Kansas, Nebraska and West Virginia, including four mule deer and three white-tailed deer (details are below in the Chronic wasting disease section).

Toxicoses and Toxicant Detection

Toxicants were detected in investigations of individual deaths as well as multi-animal die-offs. In some cases, the detected toxicant(s) was not considered the proximate cause of death. A variety of anticoagulant rodenticide compounds were detected in 28 animals, including 12 bald eagles (four each from Florida and Georgia and one each from Arkansas, North Carolina, South Carolina, and Virginia), four golden eagles (one each from Arizona, Colorado, New Mexico, and Oregon), two bobcats from South Carolina, two eastern gray squirrels (one from Florida and one from Georgia), a red fox from Virginia, and a turkey vulture from Arkansas. Lead was detected in tissues from 13 animals, including ten bald eagles (four from Georgia, two from Mississippi, and one each from Arkansas, Florida, North Carolina and Virginia), a golden eagle from Wyoming, and two American alligators from Florida. Ten of these were diagnosed with lead toxicosis. Both anticoagulant rodenticides and lead were detected in six animals. Insecticides were responsible for deaths of four white-tailed deer in Georgia (aldicarb, a carbamate) and two European starlings in Missouri (organophosphate). In addition, seven common grackles in Tennessee died of suspect cholecalciferol (vitamin D) toxicosis. Presumed ethanol toxicosis was diagnosed in two cedar waxwings in Florida and suspect pyrrolizidine alkaloid toxicosis in a white-tailed deer in Louisiana. Methylxanthines (caffeine, theobromine, and theophylline) were detected in nine aquatic birds (eight white ibis and one yellow-crowned night heron) in Florida. As mentioned below under “Bald eagles” section, multiple toxicants (aldicarb, carbofuran, acephate, cocaine, and imidacloprid) were detected in three bald eagles in close proximity in Georgia.

Four bobcats in Florida were diagnosed with lesions consistent with feline leukoencephalomyelopathy. The cause of this condition is unknown; however, samples from these and other bobcats are undergoing testing for numerous toxicants as well as vitamin A levels.

Neoplasia

Neoplasia (tumors) was diagnosed in fifteen individuals; ten of these were white-tailed deer. Tumors in white-tailed deer included cutaneous (skin) fibromas (one deer in Kansas and one in North Carolina), brain tumors (oligodendroglioma in a deer from Louisiana and astrocytoma from a deer in North Carolina), bone tumors (osteochondroma in a deer from North Carolina and osteosarcoma in a deer from Alabama), squamous cell carcinoma of the face (Georgia), liver tumor (hepatic cholangiocarcinoma; Georgia), and spindle cell carcinoma in lung (presumed metastasis; Maryland). Additional diagnoses included adenocarcinoma of lung and liver (metastatic) in a black bear from North Carolina, liver tumors in a Canada goose from North Carolina and a Virginia opossum from Nebraska (hepatocellular carcinoma and biliary carcinoma, respectively), round cell tumor (metastatic) in a wild turkey from North Carolina, and oviductal adenocarcinoma in a Puerto Rican parrot.

Bald eagles

Among 50 bald eagles evaluated (18 from Georgia, nine from Florida, eight from Arkansas, three from North Carolina, two each from Mississippi and Oklahoma, and one each from Iowa, Kansas, Kentucky, South Carolina, and West Virginia), 19 were diagnosed with trauma (including three attributed to vehicular collision and one to gunshot), and three with electrocution. Emaciation was diagnosed as the primary cause of death in two eagles. Fifteen were diagnosed with toxicosis (including presumptive), including seven with lead toxicosis, five with anticoagulant rodenticides, and three subadults from the same site in Georgia with aldicarb, carbofuran, acephate, cocaine, and imidacloprid (a neonicotinoid) detected on feathers (the fifteen eagles with toxicosis are also included in the above section, “Toxicoses and toxin detection”).

Chronic Wasting Disease

CWD Consultative Services

SCWDS continues to assist state and federal wildlife management and animal health agencies as they developed policies, programs, and regulations to prevent or manage CWD and associated risks. SCWDS assistance involves providing technical expertise regarding the status of CWD in North America to policy makers, wildlife managers, and regulatory agency authorities as they consider approaches to preventing, responding to detection, or managing CWD. This includes assisting agencies as they considered proposed regulations, program standards, and other guidelines designed to reduce the risk of the further spread of CWD and with development and refinement of CWD surveillance and management strategies. SCWDS also provides technical assistance to non-governmental organizations engaged in efforts to educate the public and policy makers about the risks that CWD poses to free-ranging cervid populations.

During the past year, SCWDS has provided frequent consultative services over phone and email. In addition, SCWDS faculty participated in a meeting between SCDNR and cervid industry representatives to provide technical expertise regarding regulatory decisions surrounding urine-based attractants, as well as attended the AGFC February Monthly Commission Meeting to participate in discussions on AGFC CWD research and management activities. Mark Ruder is a participant in the Multistate CWD Research Consortium (*North American Interdiscplinary CWD Research Consortium*) led by Michigan State University. SCWDS faculty assisted with drafting and editing the First Supplement to the AFWA Best Management Practices for Surveillance, Management and Control of CWD.

CWD Diagnostic Services

Surveillance for chronic wasting disease (CWD) of cervids from within the Cooperativecontinued. Cervids tested for CWD by SCWDS typically are submitted to the Diagnostic Service as mortality investigations, or are part of research projects. During this year, CWD testing was completed on tissue samples from 340 cervids (190 white-tailed deer (WTD), 107 mule deer (MD), 40 elk, 2 pronghorn antelope and 1 sika deer by ELISA or IHC tests. ELISA testing was performed by the Athens Veterinary Diagnostic Laboratory (AVDL), and IHC by the National Veterinary Services Laboratory (NVSL). Initial reactors on ELISA test are typically sent to NVSL for confirmation using IHC.

CWD ELISA and/or IHC Test Results

State	**WTD** (pos/n)	**Mule deer** (pos/n)	**Elk** (pos/n)	**Antelope** (pos/n)	**Sika deer** (pos/n)	**Total** (pos/n)
AR	2/11	0	0	0	0	2/11
GA	0/30	0	0	0	0	0/30
KS	3/11	8/27	0/1	0/1	0	11/40
KY	0/53	0	0/25	0	0	0/78
LA	0/1	0	0	0	0	0/1
NC	0/5	0	0	0	0	0/5
NE	4/12	12/80	4/11	0/1	0	20/104
OK	0/1	0	0/1	0	0	0/2
SC	0/6	0	0	0	0	0/6
IL (SC)*	0/1	0	0	0	0	0/1
KS (SC)*	1/2	0	0	0	0	1/2
TN	7/17	0	0	0	0	7/17
WV	7/40	0	0/2	0	0/1	7/43

* *Interstate movement of cervid parts - state of origin (state submitting samples)*

Since October 2002, SCWDS has tested 111,226 ruminant samples for CWD using IHC or ELISA, yielding 295 CWD-positive animals. These samples include active surveillance and passive surveillance specimens.

CWD Research

SCWDS initiated a project in collaboration with Colorado State University, Arkansas Game and Fish Commission, Tennessee Wildlife Resources Agency, and West Virginia Division of Natural Resources to investigate the potential for vertical transmission of CWD prions. The project is funded by the Multistate Conservation Grant Program. The overall project goal is to determine if *in utero* transmission of CWD occurs in free-ranging white-tailed deer naturally infected with CWD. Sub-objectives are to examine the distribution of prions in deer tissues, excreta, and bodily fluids, and to compare observed CWD prevalence using traditional diagnostic assays (i.e., ELISA and IHC) vs. amplification assays (i.e., RT-QuIC). To accomplish this, naturally infected, pregnant does were lethally collected in highly CWD-endemic regions of the Southeast. During early 2020, SCWDS and agency personnel collected 31 white-tailed deer for this project, including four deer from Georgia (negative control site), as well as 27 deer from CWD endemic regions of Arkansas (n=11) and Tennessee (n=16). Plans to collect in West Virginia were postponed due to the COVID-19 pandemic. Field necropsies were performed and excreta/tissue samples were carefully collected to avoid cross-contamination. Screening ELISA tests on retropharyngeal lymph nodes (RPLN) yielded 2 CWD-positive, pregnant doe from Arkansas, as well as 5 CWD-positive, pregnant doe and 2 CWD-positive bucks from Tennessee. All tissue/excreta samples collected at each field site (Arkansas, Georgia, and Tennessee) were shipped to collaborators at the CSU

Prion Research Center for further analysis. Samples from all 31 deer included: blood, urine, feces, saliva, ear skin, tarsal gland, parotid salivary gland, RPLN, obex, tonsil, lumbar lymph node, skeletal muscle, bone marrow, +/- testicle, +/- mammary gland, +/- intact uterus. Tissue samples were trimmed for histology and IHC testing, including third eye lid, RAMALT, RPLN, obex, and tonsil. Sample processing and testing is currently underway at the CSU Prion Research Center.

West Virginia Division of Natural Resources (WVDNR) and SCWDS are collaborating to analyze 14 years of CWD monitoring data in white-tailed deer from the endemic region of West Virginia. Since 2005, WVDNR has measured apparent prevalence of CWD in central Hampshire County. This is an important long-term CWD data set in the eastern United States and descriptive analysis and disease ecology modeling of this data will yield information relevant to the region. As expected, the CWD prevalence increased annually but surprisingly a statistically significant difference in CWD prevalence was not detected between the sexes. Additional analysis of the data is underway to describe this unqiue dataset from an eastern white-tailed deer population.

A large, multi-year project to understand the population-level impacts of CWD on white-tailed deer in Arkansas will be initiated by Arkansas Game and Fish Commission and the University of Georgia in the coming year. The project is lead by researchers at the Warnell School of Forestry and Natural Resources (University of Georgia) and scientists at SCWDS and Colorado State University are collaborating on the disease aspects of the project. Coordination and planning are underway ahead of the first capture season during the winter of 2021.

Herd Health Evaluations

Deer herd health evaluation services and/or final reports were provided to fish and wildlife management agencies within the southeastern region where health problems were suspected, or data were needed to make controversial deer management decisions. Final reports regarding abomasal parasite counts, histological examinations, and/or laboratory procedures, including serologic testing for antibodies to domestic ruminant pathogens, were provided to the following agencies:

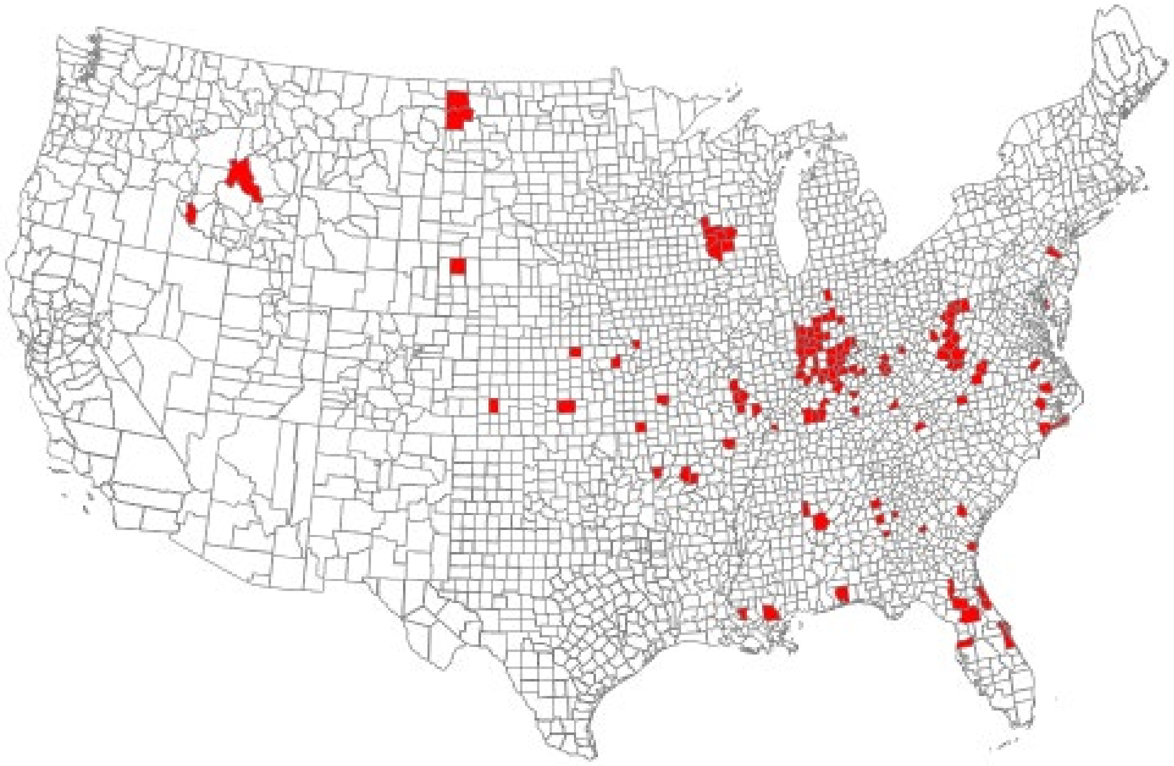
- North Carolina Wildlife Resources Commission: 5 deer collected from Cowan’s Ford Wildlife Refuge in Mecklenburg County.
- Oklahoma Department of Wildlife Conservation: 55 deer collected multiple counties, including Adair, Delaware, Ottawa, and Sequoyah Counties.
- Tennessee Wildlife Resources Agency: 31 deer collected from multiple locations, including Enterprise South Nature Park in Hamilton County, Hiwassee Refuge Wildlife Management Area in Meigs County, and Yuchi Refuge Wildlife Management Area in Rhea County.
- West Virginia Division of Natural Resources: 30 deer collected from multiple locations, including Fishing Creek in Marshall County, Garrett/Hull Farms in Lewis County, Somerville Fork in Wirt County, Cook Farm in Hardy County, Flying W Farm in Mineral County, and Slanesville in Hampshire County.

Hemorrhagic Disease

2019 HD Diagnostics: Most of the hemorrhagic disease (HD)-related work during this year was dedicated to epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) diagnostics. For the 2019 HD season, 398 submissions were received from 26 states. These included samples from 365 white-tailed deer, 16 elk, 10 mule deer, and 4 pronghorn. Three cows associated with an outbreak of EHDV-2 in West Virginia also were tested. Samples were submitted from Alabama, Alaska, Arkansas, Delaware, Florida, Georgia, Idaho, Indiana, Kansas, Kentucky, Louisiana, Maryland, Michigan, Mississippi, Missouri, Montana, Nebraska, New

Jersey, North Carolina, North Dakota, Pennsylvania, South Carolina, Tennessee, Virginia, West Virginia, and Wisconsin. A total of 214 virus detections were made using serogroup-specific RT-PCR. Positive cases were then tested by virus isolation for EHDV and BTV. A total of 140 viruses were isolated and serotyped, including EHDV-1 from Georgia and EHDV-2 from Alabama, Arkansas, Idaho, Indiana, Kansas, Kentucky, Louisiana, Maryland, Missouri, North Carolina, North Dakota, Virginia, and West Virginia. BTV-2 was isolated from Georgia, and BTV-13 was isolated from Florida. With the exceptions of positive pronghorn from Idaho, elk from Kentucky, mule deer from Kansas and Idaho, and cattle from West Virginia, all virus detections came from white-tailed deer.

Counties with EHDV and/or BTV detections (virus isolation and/or RT-PCR) made by SCWDS during the 2019 trans mission season.



Drought as a potential risk factor for HD: A collaborative project between SCWDS and Michigan State University was published this fiscal year describing the potential for drought to serve as a risk factor for severe HD outbreaks. There are many potential environmental, host, and viral factors that interact to produce and drive an HD outbreak. Climate factors, such as temperature, not only influence vector population dynamics but can also affect the ability of EHDV and BTV to replicate in these vectors. Drought, which is often correlated with high temperature along with low seasonal rainfall events, is another example of a potential risk factor that may not only affect *Culicoides* development but may also have host related implications such as increasing host density around water sources and wetland areas where *Culicoides* vectors breed. During 2007 and 2012, widespread HD outbreaks occurred in the eastern and midwestern United States and there was a spatial correlation between areas affected by severe drought and areas where HD occurred. Previous research also has identified drought like conditions, such as high temperatures and low precipitation, as risk factors for HD. To further investigate this potential relationship, we focused on 23 states in the eastern United States that have reported HD related mortality during the period 2000-2014. These included states where HD has historically occurred since 1980 and states such as Michigan, Pennsylvania, and Wisconsin where HD reports have been recently documented or are increasing. The long-term data obtained from the annual SCWDS HD Questionnaire was used in this analysis. The period 2000-2014 was selected based on the availability of data from the US Drought Monitor. In addition to HD mortality and drought data, other potentially relevant drivers and factors were included in the analysis; these included, wetland cover, physiographic region, latitude, longitude, year, and state. The analysis was done using a generalized linear mixed model to explain HD occurrence based on these potential spatiotemporal predictors. Results indicated that drought severity was a significant predictor of HD presence. This relationship, however, was highly dependent on latitude and only was significant at northern latitudes. Overall, the findings improve our understanding of the epidemiology of HD and help to inform deer management but also highlight the regional variation in epidemiologic patterns of HD and the complexity of this vector-borne disease system.

Surveys for *Culicoides* spp. in the Southeastern U.S.

Biting mides of the genus *Culicoides* are tiny blood feeding flies and are vectors of EHDV and BTV. At least 150 species of Culicoides occur in North America, although only a small number are vectors. Since 2008, SCWDS has been conducting surveillance for native and exotic *Culicoides* spp. across the southeastern U.S., with partial funding through a Cooperative Agreement with USDA-APHIS-Veterinary Services. SCWDS conducts surveys of adult midges using CDC UV light traps to collect insects from chosen field sites. The surveys focus on identifying potential and confirmed vectors of EHDV and BTV, but also capture general *Culicoides* species complex composition across a range of habitats. These surveys have shed light on the ecology, distribution, and seasonal dynamics of *Culicoides* spp. in the Southeast. After years of a large-scale regional survey, we are now conducting fine-scale, local surveys in Athens, GA.

Local Surveys

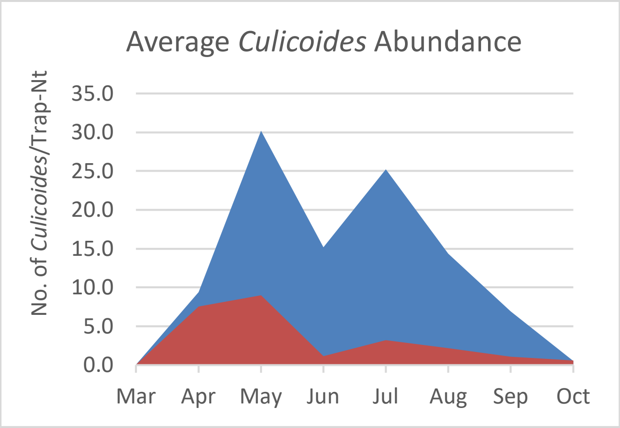
A main factor in viral spread—vector dispersal—depends on the seasonal density and population dynamics of those vectors, as well as available habitat types and other environmental factors and land-use patterns (e.g., agricultural and forestry practices). Despite this understanding, there are still large knowledge gaps in these areas of *Culicoides* ecology, as well regarding what species are serving as vectors of BTV and EHDV, particularly in the southeastern U.S. where *C. sonorensis*, the presumed vector, is rare except in areas heavily influenced by livestock. To begin to address these gaps, two local sites with distinct land and animal use patterns were chosen at which to conduct insect surveys. Site 1 is predominated by natural and managed Piedmont forest habitats with abundant native wildlife and a captive white-tailed deer herd; Site 2 is a dairy with several hundred head of Holstein cattle and associated pasture, stall, pond, and forest edge habitats. Sites have been surveyed monthly from March through October since July 2016 (Site 1) or July 2018 (Site 2). The two Sites are located approximately six miles apart.

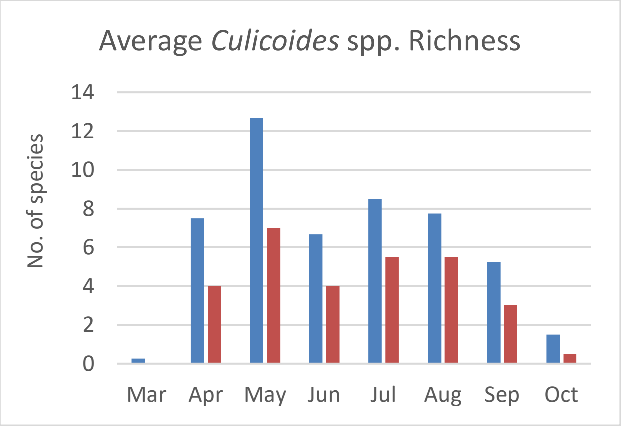
Overview: Site 1 and Site 2

SITE 1 (FOREST)
JULY 2016 – JUNE 2020
40 SURVEY NIGHTS
379 TRAP-NIGHTS
28 SPECIES COLLECTED

SITE 2 (DAIRY)
JULY 2018 – JUNE 2020
20 SURVEY NIGHTS
120 TRAP-NIGHTS
13 SPECIES COLLECTED

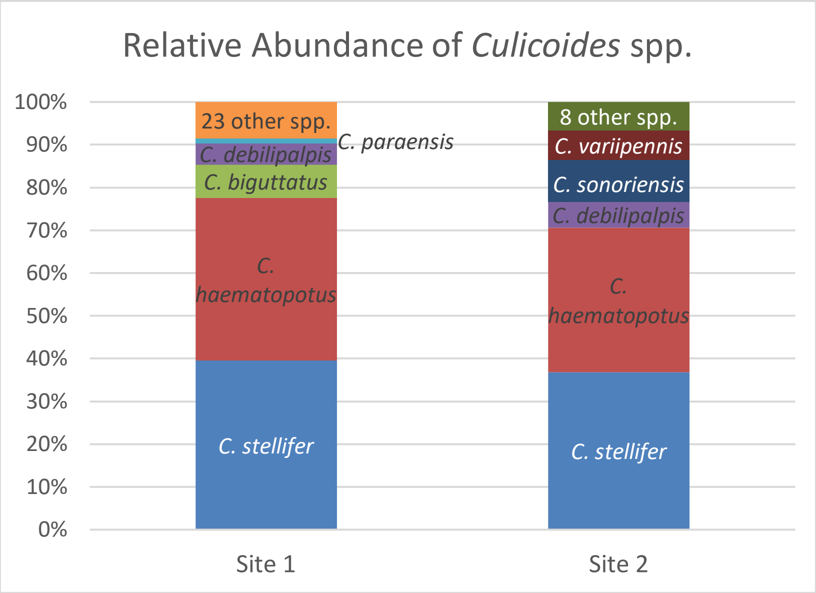
- Throughout the trapping year, traps at Site 1 consistently collected more *Culicoides* individuals (abundance) and more *Culicoides* species (species richness) than at Site 2. (See Figures below)



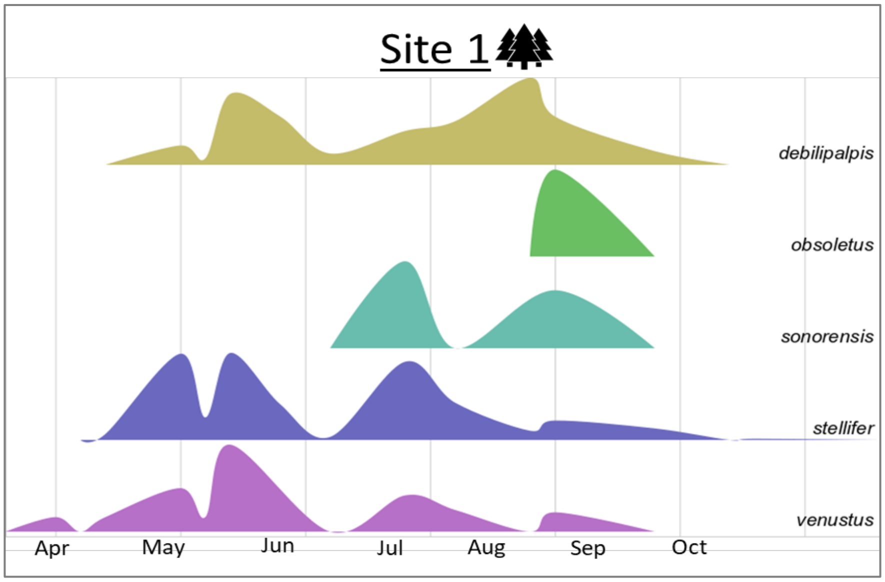


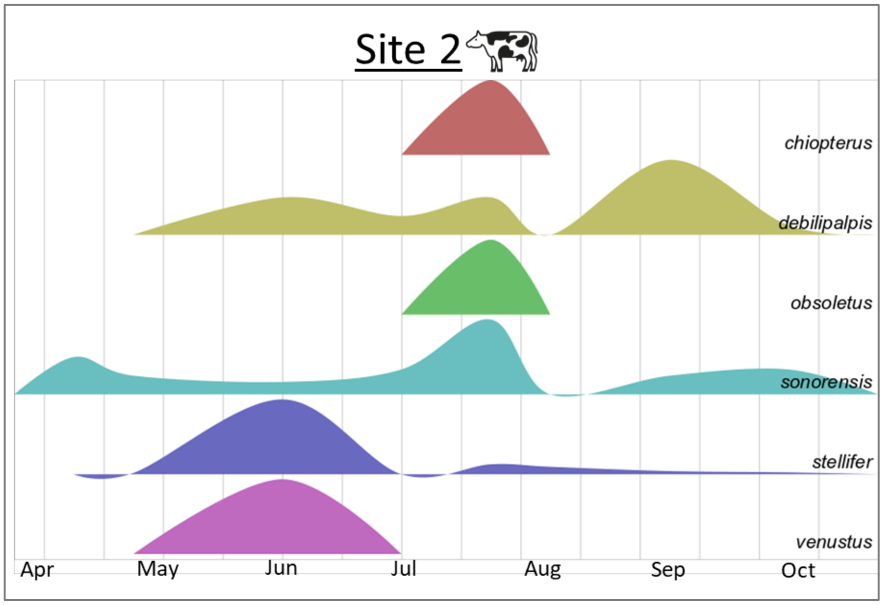
Site 1 vs. Site 2

- At both Sites, *C. stellifer, C. haematopotus*, and *C. debilipalpis* were the most abundant species. (See Figure)
- At Site 1, *C. biguttatus* and *C. paraensis* were also among the most abundant species.
- At Site 2, *C. sonorensis* and *C. variipennis* were also among the most abundant species. These species are known to be livestock-associated.



Comparison of phenology of Culicoides vectors of EHDV/BTV by site





- At both Sites, known** and suspected* EHDV/BTV vector species collected included: *C. debilipalpis*, C. obsoletus*, C. sonorensis**, C. stellifer*, and C. venustus*.* At Site 2, the suspected vector species *C. chiopterus* was also collected. *C. sonorensis*, the primary known vector of BTV/EHDV in North America was present at both Sites, however, it was collected at Site 2 in higher numbers, in more traps, and during more months than at Site 1 (see Figures above and Table below).
- The phenology of *C. stellifer* and *C. venustus* varied between Sites: At Site 1, both species were present throughout the year; however, at Site 2, they were primarily present only in the spring and early summer. *C. debilipalpis* was present starting in late spring through early fall at both Sites. *C. obsoletus* and *C. chiopterus* (known vectors in Europe) were only present in mid- to late summer (see Figures).

- Publications describing the work are in preparation and future work will involve identification of species specific larval development sites within these two study locations.

Comparison of C. sonorensis detections between Site 1 (natural area) and Site 2 (dairy)

	TOTAL *CULICOIDES*	NO. OF TRAP-NTS	NO. OF SURVEYS	MONTHS PRESENT
SITE 1	4 (0.1%)	4 (1%)	4 (11%)	Jul (2017, 2018), Aug (2016, 2019)
SITE 2	22 (10%)	15 (17%)	8 (53%)	Apr (2019, 2020), May (2019), Jun (2019), Jul (2018), Aug (2018), Sep (2018, 2019)

Additional Culicoides Activities

- Data from SCWDS' regional *Culicoides* spp. surveys in the southeastern United States, which were ongoing from 2008 to 2018 and spanned nine states, are being used to support model development through collaborations with USDA-APHIS-Center for Epidemiology and Animal Health (CEAH) and the University of Melbourne, Australia. The project aims to parameterize the Australian Animal Disease Spread (AADIS) model for simulation of vector-borne disease spread in the US. SCWDS staff provide expertise on *Culicoides* spp. distribution and ecology, and BTV transmission and surveillance.
- SCWDS continues to offer assistance to any state and/or federal agency partners with *Culicoides* surveillance, particularly in response to EHDV/BTV outbreaks. During summer/fall of 2019, SCWDS partnered with the Kansas Department of Wildlife, Parks, and Tourism (KDWPT) to conduct *Culicoides* surveys at locations of HD outbreaks. SCWDS sent light traps to KDWPT to conduct surveys, and insect collections were submitted to SCWDS for *Culicoides* spp. identification (pending).
- SCWDS continues to offer expertise in the area of *Culicoides* spp. identification to domestic and international organizations. This year, SCWDS staff provided consultative support to projects by the Canadian Food Inspection Agency and the University of Guelph. Publications stemming from these projects are in preparation.

Surveys for Cattle Fever Ticks on Wildlife in Texas

Native white-tailed deer and some non-native invasive species, such as nilgai, are important hosts of cattle fever ticks (*Rhipicephalus annulatus* and *R. microplus*), and their presence on the landscape complicates the efforts of the USDA's Cattle Fever Tick Eradication Program (CFTEP). To support the activities of the USDA and the Texas Animal Health Commission (TAHC), SCWDS provides enhanced surveillance of wildlife to help inform the CFTEP. In coordination with Texas Parks and Wildlife Department (TPWD) and U.S. Fish and Wildlife Service (USFWS), SCWDS has been conducting cattle fever tick surveys of free-ranging wildlife. These activities help fill surveillance gaps, identify research needs, and potentially aid in the evaluation of quarantine and treatment procedures. Additionally, surveillance can be targeted in areas outside the Permanent Quarantine Zone from which tick-infested animals have been identified. All tick collections are submitted to National Veterinary Services Laboratories (NVSL) for identification.

- During December 2019 and January 2020, SCWDS personnel conducted passive cattle fever tick surveys via examination of hunter-harvested white-tailed deer and other native and exotic ungulates at animal processing facilities in and around Edinburg, Texas. Ticks were collected from animals harvested from 13 south Texas counties including: Bexar, Brooks, Duval, Hidalgo, Jim Hogg, Jim Wells, Kenedy, Live Oak, Starr, Upton, Webb, Willacy, and Zapata. No cattle fever ticks were collected. All other tick collections were submitted to NVSL for species identification. Results will be reported when they are received from NVSL. Additional surveys are planned for December 2020 and January 2021. See Tables and Figure below for additional information.

Hunter-harvested animals examined by SCWDS in Dec 2019-Jan 2020, by species.

HOST SPECIES	N	W/ TICKS	% TICK INFESTED
WHITE-TAILED DEER	214	76	36%
NILGAI	10	6	60%
MULE DEER	3	2	67%
ELK	2	0	0%
AXIS DEER	1	1	100%
SCIMITAR ORYX	1	1	100%
TOTAL	**231**	**86**	**37%**

Hunter-harvested animals examined by SCWDS in Dec 2019-Jan 2020, by Texas County.

TX COUNTY	N	W/ TICKS	% INFESTED
UPTON	2	2	100%
WILLACY	2	2	100%
BEXAR	1	1	100%
KENEDY	5	3	60%
WEBB	2	1	50%
UNKNOWN	18	8	44%
HIDALGO	51	22	43%
BROOKS	22	9	41%
DUVAL	16	6	38%
STARR	91	31	34%
JIM HOGG	12	1	8%
JIM WELLS	2	0	0%
LIVE OAK	1	0	0%
ZAPATA	6	0	0%

- SCWDS personnel planned to assist researchers at Texas A&M University-Kingsville on a large, multidisciplinary project involving free-ranging white-tailed deer collections near the Falcon Reservoir in Starr and Zapata Counties along the US-Mexico border. SCWDS staff planned to provide technical expertise and assistance in animal examination, tick infestation assessment, tick collection, and tick identification during the initial capture period (February 2020) and the targeted culling (March 2020). Due to USDA-ARS permitting concerns and travel restrictions associated with the COVID-19 pandemic, SCWDS was not able to participate in this work.

- Final preparations are underway for active cattle fever tick surveillance by lethal collection of nilgae from Laguna Atascosa National Wildlife Refuge. The goal of the work will be to better understand cattle fever tick infestation rates and burdens on this invasive species during anticipated the summer months. The intention is for this to be a long-term, annual effort, that can provide additional information and help inform mitigation strategies and understand tick ecology. Collections are scheduled for August and September 2020.

Surveys for Asian Longhorned Tick in the Eastern U.S.

Haemaphysalis longicornis, the Asian longhorned tick (ALT), is native to East Asia but was recently introduced to the United States and has now become established. Cattle are an important host for ALT, but this tick has also been found on a wide range of other domestic animal hosts, as well as humans and wildlife in the U.S. Additionally, it is a confirmed or suspected vector of a variety of human and animal pathogens in other regions, making it a potential health threat to humans and animals in the U.S.

In an effort to help determine geographic range of ALT in North America, as well elucidate host range and preference, and potential disease risks, SCWDS is conducting surveys for ticks in the eastern U.S. These surveys have been ongoing since fall 2017, shortly after ALT was initially discovered in the U.S. Active surveys in areas with known ALT infestations are conducted by trapping and examining free-ranging birds and mammals for ticks, as well as through tick drags and traps. Passive surveillance has been accomplished by coordinating a large, multi-state network of state wildlife agencies and wildlife rehabilitation centers that have agreed to collect ticks from targeted species and relay them to SCWDS for identification. This method, utilizing multiple levels of surveillance, has allowed SCWDS to examine ticks from 315 tick drags/traps and ~1620 individual animals from 22 states. North American animal species serving as hosts for ALT in seven states (KY, MD, NC, NJ, PA, VA) identified though SCWDS surveillance include: coyote, domestic dog, eastern cottontail, elk, gray fox, raccoon, red fox, red-tailed hawk, Virginia opossum, white-tailed deer, and woodchuck.

All ticks collected during both active and passive surveys are initially identified by morphological methods at SCWDS. Identification of ticks determined to be ALT may be confirmed using molecular methods. If a specimen represents a new location and/or host species record, it is sent to NVSL for species identification confirmation.

From July 2019 to June 2020:

- Passive Surveillance: State wildlife agencies and wildlife rehabilitators have been participating in *H. longicornis* surveillance by submitting tick specimens collected from wildlife to SCWDS. Since 2017, we have received ~1110 tick samples from wildlife agencies and ~250 tick samples from wildlife rehabilitators collected from 30 host species across 22 states. Our surveillance detected ALT infestations on 26 individual cervids (white-tailed deer and elk) from 5 states (KY, MD, NC, PA, WV), 5 canids (red fox and domestic dog) from NC and VA, and from a black bear from KY. SCWDS continued to send out tick collection supplies to cooperating wildlife agencies and wildlife rehabilitators as needed.
- Active Surveillance: In May, July, and September 2019, SCWDS personnel conducted small and mesomammal surveys and environmental sampling (100m tick drag transects) on a cattle farm in Albemarle County, Virginia, a location with known ALT infestation affecting cattle in 2018. Tick drags were conducted in a variety of habitats to better understand ALT habitat associations. During 2019, 238 transect drags (100m each) were conducted, yielding 413 ticks, 239 of which were ALT. Asian longhorned ticks were detected in all three habitat types (pasture, edge, forest). Ninety nine wild mammals trapped and examined for ticks, yielding 404 ticks of various species. Five wildlife species were infested with ALT (see Table below).

SCWDS ALT Surveillance; Albemarle County, Virginia, 2019.

	HOST SPECIES/SOURCE	N	W/ TICKS	W/ ALT	% W/ ALT
SPRING	*Peromyscus* sp.	41	22	0	0%
MAY 2-8, 2019	Hispid cotton rat	1	0	0	0%
	Gray squirrel	1	0	0	0%
	Garter snake	1	0	0	0%
	Total hosts, spring	**44**	**22**	**0**	**0%**
	Tick drag, spring	**64**	**31**	**29**	**45%**
SUMMER	*Peromyscus* sp.	17	1	0	0%
JULY 9-21, 2019	Gray squirrel	2	1	0	0%
	Eastern woodrat	1	0	0	0%
	Woodchuck	2	1	1	50%
	Eastern cottontail	1	1	0	0%
	Virginia opossum	6	6	1	17%
	Raccoon	9	9	4	44%
	White-tailed deer	1	1	1	100%
	Human	1	1	0	0%
	Total hosts, summer	**40**	**21**	**7**	**18%**
	Tick drag, summer	**110**	**25**	**18**	**16%**
FALL	*Peromyscus* sp.	9	1	0	0%
SEPT 5-11, 2019	Eastern woodrat	1	0	0	0%
	Coyote	2	2	2	100%
	Human	2	2	1	50%
	Total hosts, fall	**14**	**5**	**3**	**21%**
	Tick drag, fall	**64**	**33**	**30**	**47%**

- Active Surveillance: In May 2020, SCWDS conducted another round of ALT surveillance in Albemarle County, Virginia. Ticks were collected through environmental sampling, and through the examination of free-ranging rodents(see Table below):

SCWDS Tick Surveillance; Albemarle County, Virginia, May 2020

Host Examined/ Collection Method	No. w/ *ALT (%)*	Other tick spp. collected
Tick drag	**20/23 (87%)**	*Dermacentor variabilis, Ixodes scapularis, Amblyomma americanum, A. maculatum*
Peromyscus sp.	0/20 (0%)	*D. variabilis, I. scapularis, A. maculatum*
Woodland jumping mouse	0/1 (0%)	*A. maculatum*
Hispid cotton rat	0/1 (0%)	n/a
Human	0/4 (0%)	*D. variabilis, D. albipictus, I. scapularis, A. americanum*

- Active surveillance: In spring of 2020, SCWDS began conducting surveillance for ALT in north Georgia and South Carolina at several WMAs and National Forests. These surveys utilized a combination of tick drags and 100 trap-nights of mesomammal trapping. Laboratory identification of ticks collected from all drags and wildlife are ongoing. To date, no *H. longicornis* has been detected.

- Previous wildlife surveys conducted by SCWDS in 2018 identified seven North American species serving as hosts for ALT. These efforts have been detailed in a manuscript in *Transboundary and Emerging Diseases.*
- To aid in tick identification efforts, a polymerase chain reaction restriction fragment length polymorphism (RFLP) assay was previously developed. This assay has been shown to distinguish ALT from the other three *Haemaphysalis* species that occur in North America (*H. leporispalustris*, *H. chordeilis*, and *H. juxtakochi*) and is useful in the rapid identification of damaged specimens. A manuscript this assay has been published in *Frontiers in Veterinary Science*. We continue to use this assay for identification of *Haemaphysalis* species, in addition to morphological identification.
- Multiple molecular projects involving ALT are currently underway.
  - The population genetics of ALT is currently being investigated to identify possible source populations to those found in the U.S.
  - With the reports of *Theileria orientalis* Ikeda genotype from Alblemarle County, Virginia, tissue samples from white-tailed deer (blood or spleen) and field collected ticks (ALT and other tick species) are being screened for *Theileria* spp. and other pathogens using molecular techniques to understand the potential role of ALT and white-tailed deer in the epidemiology of this pathogen. To date, >350 cervid blood/spleen samples from the eastern U.S. have been testing and >80% were positive for WTD *Theileria* sp. (often called *T. cervi*) and ~8% were positive for *Babesia* spp. No WTD samples were positive for T. orientalis. Additional WTD samples are being acquired for testing. Further, field-collected ticks from the index site of the *T. orientalis* Ikeda outbreak (Virginia) have been tested and 13% (15/118) of ALT were positive for *T. orientalis* Ikeda. Other pathogens detected in these ALT include (*Rickettsia felis* (n=1) and *Anaplasma phagocytophilum* (n=1). Of the 28 *Amblyomma americanum* and 10 *Dermacentor variablis* collected from this same site, none tested positive for *T. orientalis*. Additionally, 96 ALT collected from New Jersey (a location without livestock) tested negative for *T. orientalis*. This work was published this year in *Ticks and Tick-borne Diseases*.
- SCWDS maintains a website that is accessible to the public, which highlights basic information about ALT, SCWDS' tick surveillance program, hosts from which ALT has been recorded, and a map with known ALT collections. This website can be accessed directly at https://scwds.shinyapps.io/haemaphysalis/ or from the SCWDS website (https://vet.uga.edu/scwds/). SCWDS also maintains a database that is not accessible to the public, which stores all ectoparasite collection information gathered over the past two decades through SCWDS wildlife surveillance.
- All ALT surveillance data collected by SCWDS is made available to the USDA on a regular basis to be included in the National *Haemaphysalis longicornis* Situation Report, which is published periodically.

Bear Mange

A study continued to investigate the unprecedented increase in the number and geographic distribution of severe mange cases in black bears in Pennsylvania and surrounding states. Previous work with the Pennsylvania Game Commission investigated different diagnostic assays for detection and identification of the mite(s) associated with mange cases and found that all of the cases were due to *Sarcoptes*. Additionally, we described the spatial and temporal aspects of the emergence and expansion of sarcoptic mange in black bears in the eastern United States. We continue to receive samples from wildlife species with lesions consistent with mange to determine the mite species present. During this past year we finalized several publications and prepared to initiate a new population genetic study on *Sarcoptes* mites from wildlife.

A study on coinfections of bears with *Sarcoptes* and other pathogens (e.g., canine parvovirus, canine adenovirus, canine distemper virus, *Trichinella* spp., and *Toxoplasma gondii)* was completed and a manuscript published in the journal *Veterinary Sciences (open access at* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6958396/*)*. The results indicate that bears with mange are no more likely to have antibodies to any of these pathogens than clinically normal bears.

We completed a study that explored the utility of serology in sarcoptic mange surveillance in bears in Pennsylvania and published a manuscript in the *Journal of Wildlife Diseases*. Briefly, we validated the use of a commercial assay for bears and although the kit was designed for use in dogs, it showed a high sensitivity and specificity. To further examine the performance of this assay, serial serum samples from black bears with confirmed sarcoptic mange were collected post-treatment to determine the persistence of detectable antibody response with the ELISA. These samples were collected by collaborators at The Wildlife Center of Virginia. Antibodies in bears waned to below the limit of detection between four and fourteen weeks suggesting that serology studies may under-estimate the number of exposed bears after antibodies have waned. State-wide serosurveys in Pennsylvania from hunter-harvested bears over two years showed a significant difference in seroprevalence between regions with high mange (6.7%) and low mange (no seropositive bears were detected). Within Pennsylvania, these data indicate that the geographic distribution of exposure to *S. scabiei*, based on serologic testing, generally reflects the distribution of overt disease, as determined by syndromic surveillance. Collectively, these results indicate the evaluated ELISA is an effective tool for monitoring *S. scabiei* exposure in bear populations and provides the framework for additional studies regarding mange epidemiology in bears.

Because *S. scabiei* can be transmitted to bears from environmental sources or other infested items (e.g., traps), data on environmental persistence is important for guiding management and public communications. Thus, we wanted to investigate how long mites could remain alive when off of the bear host. We investigated this through a laboratory experimental study. Full section skin samples and superficial skin scrapes were collected from bears immediately after euthanasia due to severe mange. After ~ 24 h on ice packs (shipment to lab), samples were placed in dishes at 0, 4, 18, or 30°C and 60, 20, 12, and 25% relative humidity, respectively, and the percentage of mites alive, by life stage, was periodically determined. Humidity was recorded but not controlled. Temperature significantly affected mite survival, which was shortest at 0°C (mostly ≤4 h) and longest at 4°C (up to 13 days). No mites survived beyond 8 days at 18°C or 6 days at 30°C. Mites from full-thickness skin sections survived significantly longer than those from superficial skin scrapes. Adults typically survived longer than nymphs and larvae except at 30°C where adults survived the shortest time. These data indicate that at cooler temperatures, *S. scabiei* can survive for days to over a week in the environment, especially if on host skin. However, these data also indicate that the environment is unlikely to be a long-term source of *S. scabiei* infection to bears, other wildlife, or domestic animals. A manuscript describing these findings was published (open access) in the journal *Parasitology Research*.

Finally, we collaborated with colleagues from Tufts University to describe the first case of chorioptic mange in a black bear from Massachusetts. The bear presented with patchy hair loss and crusting over the entire body was but most pronounced on the muzzle, ears and hind legs. After treatment with selamectin, low numbers of mites were observed in skin crusts. Morphologically, the mites were a *Chorioptes* sp. Sequence analysis of the ITS-2 region confirmed they the mites were a *Chorioptes* sp. and that it grouped with a *Chorioptes* sp. collected from a panda in China. This report is the first to suggest that a *Chorioptes* sp. can cause mild skin lesions consistent with mange in a black bear. As mange is an increasingly important disease in black bears, this case emphasizes the importance of performing appropriate skin scrapes and mite identification in animals with hyperkeratotic skin lesions because of the clinical similarities of sarcoptic, ursicoptic, chorioptic, and to a lesser extent, demodectic mange. Confirming the disease as well as the causative agent in these cases is important as these mite species have different health implications for humans, bears, as well as other wild and domestic animals.

Investigation of feline leukomyelopathy (FLM) in bobcats and Florida panthers

SCWDS continues to assist FWC in their investigation of FLM in bobcats and Florida panthers. Since 2017, FWC has been investigating an emerging neurological disorder affecting bobcats and panthers in widespread regions of Florida. Affected animals often present with hind limb weakness and incoordination, and become emaciated with time. Full gross and histological examination of wild felids is performed at SCWDS, with careful dissection and examination of the central nervous system (brain and spinal cord). Additionally, tissues are occasionally received from field necropsied felids. Further, SCWDS has reached out to surrounding states to further inform the potential geographic distribution of FLM. From 2018-2020, SCWDS has received 24 bobcats and one Florida panther from Florida for examination. Microscopically, the most consistent lesion in animals with FLM is symmetrical white matter degeneration in the spinal cord with dilated myelin sheaths, and shrunken or absent axons. Pathological characterization of this emerging disease and investigations of potential causes are ongoing. To date, bobcats examined from surrounding states have not had lesions consistent with FLM.

Baylisascaris procyonis

Projects continue to investigate the ecology and epidemiology of *Baylisascaris* infections in raccoons and other hosts.

We continued collaborating with IDEXX Laboratories to assess the utility of a novel ascarid coproantigen ELISA for detection of intestinal *Baylisascaris* infections (from raccoons and dogs). In addition, we are interested in the role that dogs may serve as hosts for *Baylisascaris*. Domestic dogs can function as either paratenic or definitive hosts for the parasite. However, how often a dog develops a patent infection after exposure is poorly understood. Our goal was to compare the infection dynamics of *B. procyonis* in dog and raccoon hosts, including pre-patent periods, egg output, and infection efficiency. We tested fecal samples with centrifugal fecal flotation as well as the IDEXX coproantigen ELISA that can detect canine and feline ascarid (*Toxocara* spp. and *Toxascaris leonina*) infections prior to patency based on egg shedding. Groups of 12 six-month-old dogs and three-month-old raccoons were orally inoculated with larvated *B. procyonis* eggs or were fed laboratory mice that were orally inoculated with *B. procyonis* eggs. Only two dogs developed patent infections and all 12 raccoons became infected, although two raccoons did not develop patent infections. Prepatent periods were shorter in raccoons compared with the two dogs that became patent. Maximum eggs per gram of feces (EPG) was orders of magnitude greater in raccoons (up to ~10,500 EPG) compared with dogs, which did not exceed 600 EPG. The two dogs spontaneously lost infections while raccoon remained chronically infected. The coproantigen ELISA successfully detected *B. procyonis* antigens in all 12 raccoons and the 2 infected dogs. The optical density values were markedly greater in the raccoons suggesting higher intensity infections. In summary, our results demonstrate that dogs are capable of becoming patently infected with *B. procyonis*; however, they are not ideal hosts and growth and fecundity are constrained compared to the natural raccoon host. Despite dogs having low host competence in this study, patently infected dogs still pose a risk for human exposure, so measures should still be taken to minimize the exposure of dogs to *B. procyonis*. In addition, these data indicate that the commercially available IDEXX coproantigen assay for *Toxocara* can detect

Baylisascaris infections, even before they become patent. A manuscript describing these findings was published in the journal *Parasitology*.

In collaboration with the Pennsylvania Game Commission, we continue to investigate the prevalence of *B. columnaris* in skunks and *B. transfuga* in bears in Pennsylvania and will determine if the IDEXX coproantigen test for ascarids also detects *B. columnaris* and *B. transfuga* antigens. Previously fecal floats or examination of intestinal tracts were all completed and fecal samples will be tested using the coproantigen test in the future.

Raccoon Pathogen Dynamics in Anthropogenic Landscapes

We finalized the work to understand the pathogen dynamics of raccoons in anthropogenic landscapes, including by studying *Salmonella* spp. prevalence and diversity across habitats in Georgia and canine distemper virus (CDV) exposure over time in the agricultural areas of Indiana. The manuscripts are in preparation.

Salmonella in raccoons: To understand how diverse biotic and abiotic factors affect the prevalence and diversity of *Salmonella* spp. in free-ranging raccoons (*Procyon lotor*), we performed repeated cross-sectional studies across three of Georgia's ecosystems (barrier islands, Piedmont, and Coastal Plain). We 1) compared raccoon *Salmonella* prevalence and serotype diversity across urban, natural and agricultural habitats within those physiographic regions; and 2) assessed the impact of season, habitat characteristics, and demographic factors on the occurrence of *Salmonella* in raccoon fecal and paw samples. To specifically compare the impact of supplemental feeding on the *Salmonella* prevalence of raccoons, we also compared raccoons that were fed at Trap-Neuter-Return (TNR) colonies on Jekyll Island, with those that were not supplemented in a similar habitat on Wormsloe Historic Site.

Across all habitats, we sampled raccoons across three regions in Georgia and collected 114 fecal samples from 110 individuals (4 were recaptured). *Salmonella* was detected in 46% of all raccoon feces. There was no statistically significant difference between *Salmonella* occurrence in raccoon feces and raccoon breeding season, year, region, nor site. Insufficient age data for raccoons from the Piedmont and Coastal Plain prevented further analysis. As all sites were not sampled in all seasons, results for breeding season are likely confounded.

In order to determine the effects of supplemental feeding on the prevalence of *Salmonella* as a result of feral cat TNR feeding stations, we collected paw and fecal samples from raccoons captured in JI and WHS. In this comparison, *Salmonella* was detected in 35% and 38% of paw and fecal samples, respectively. We also noted that the prevalence of juveniles shedding *Salmonella* (57%) was higher, yet not statistically significantly when compared with adults (36%). Between the two barrier islands, although the prevalence was higher at WHS than at JI, the difference was not statistically significant.

We obtained 73 confirmed isolates of *Salmonella* from all sample types and sites. The 73 isolates represented 22 distinct serotypes, seven of which ranked among the top 20 in human cases in the USA, and 13 ranked in the top 20 in human cases in Georgia. Six serotypes recovered from 16 isolates from the Piedmont region included, Hartford, Give, Rubislaw, Anatum, Bareilly, and Muenchen. From the Coastal Plain we recovered 12 serotypes from 15 isolates including Bareilly, Muenster, Anatum, Arizona, Give var. 15+, Hartford, Inverness, Montevideo, Newport, PolyD, Rubislaw, and Saint Paul. Fourteen serotypes were recovered from 42 isolates from the barrier islands and included Hartford, Anatum_var._15+, Anatum, Urbana, Give, Rubislaw, Tallahassee, Albany, Bovismorbificans, Braenderup, Johannesburg, Miami, Montevideo, and Muenchen.

Twenty-eight (49.1%) of 57 raccoons captured at the barrier islands were shedding *Salmonella* in their feces and/or paws. Of these raccoons, *Salmonella* was detected on both paw and fecal samples from the same animal in 14 (50%) cases. When *Salmonella* was detected from both feces and paws, the serotype was the same in 6 (42.9%) cases, and was different in 8 (57.1%). *Salmonella* was detected in only fecal or paw samples in the remaining 14 *Salmonella*-positive animals (50%). In JI, out of 37 raccoons sampled, 17 (46%) tested positive for *Salmonella*. For 6 raccoons (35.3%) *Salmonella* was only detected in feces and for 2 raccoons (11.8%) only in the paw samples. In 9 animals, both feces and paws were positive. Of these, serotypes were the same for both feces and paws for 4 (44.4%) raccoons and was different for 5 (55.5%) raccoons. At WHS, 11 (55%) out of 20 raccoons sampled tested positive for *Salmonella*. Animals were positive for both paws and feces in 5 cases. The same serotype was detected in both feces and paws in 2 (40%) cases, and different serotypes in 3 (60%) cases. In the remaining 6 cases of *Salmonella*-positive animals, *Salmonella* was only detected in either paw or fecal samples.

We performed model selection to explore the factors that influenced *Salmonella* prevalence. The results indicated that the best models for *Salmonella* prevalence rates in raccoons included the predictors “weight” and “livestock” (33.47% support), and a model that included only the single predictor, “weight” (33.26% support). Overall, the results suggest that a large number of raccoons in Georgia can act as biological and/or mechanical carriers of *Salmonella,* and raccoon infection may vary based on factors including age, gender, and habitat characteristics.

Distemper in raccoons: We also continued work to understand canine distemper virus (CDV) transmission dynamics in raccoons. Raccoons are widespread, abundant, and well-adapted to human-altered environments and studying this species will provide a better understand the epidemiology of CDV in a changing world. Canine distemper virus (CDV) is a significant cause of mortality for animal species (wild and domestic), especially carnivores such as raccoons, gray foxes and domestic dogs. Transmission risk may increase among animals at diverse interfaces as a result of activities such as urbanization and supplemental feeding. Host demographics and landscape characteristics also potentially influence transmission. As the world’s human population increases so do urban and agricultural areas, resulting in increased human-domestic animal-wildlife interactions and potential transmission. We sampled 37 free-ranging raccoons (25 adult, 4 juveniles, and 8 yearlings – at time of first capture) multiple times between 2007 and 2010 within an agricultural ecosystem in Indiana, USA. Serum samples were tested for CDV antibodies using a serum neutralization assay. For some individuals CDV titers remained stable but in other cases titers fluctuated significantly. Age at time of initial capture was statistically significant (p=0.00389). Mean CDV antibody levels were not evenly distributed and were in fact higher in adults (<2yrs) than yearlings (1-2yrs), and higher in yearlings than juveniles (<1yr). No significant difference was noted between females and males. Mean CDV antibody titers varied significantly between capture sites (p=0.0025). Seroconversion occurred in 22% of raccoons (n=8). Five racoons (14%) had titers that were initially positive (≥4) and at levels considered protective (≥32) but which later fell below a protective level (<32). 5% of raccoons (n=2) had titers that remained positive (>4) and below the protective level. In addition, CDV antibody levels for 11% (n=4) of raccoons remained negative (<4) over multiple years. Our data show that titers fluctuate greatly within individuals as a function of age, habitat characteristics and between years. These results add to our understanding of CDV epidemiology. It also presents new data to our knowledge of immunity as a function of exposure or protection from subsequent infection. Various immunological mechanisms and other factors, such as adequate nutrition, may help protect these raccoons from clinical disease despite having low neutralizing antibody titers.

Ehrlichiosis, Anaplasmosis, and Other Tick-Borne Bacterial Pathogens

Borrelia in gamebirds: We have worked with the Pennsylvania Game Commission to investigate the prevalence and diversity of *Borrelia* in gamebirds in Pennsylvania. Lyme disease is of particular interest because the incidence in the northeastern United States continues to increase in both dogs and humans. Birds have a potentially important role in the ecology of

Borrelia species, because they are hosts for numerous tick vectors and competent hosts for various *Borrelia* spp. Four free-living species of upland game birds in Pennsylvania were tested including wild turkey, ruffed grouse, ring-necked pheasants, and American woodcock. We tested 205 tissue samples (bone marrow and/or spleen samples) from 169 individuals and detected *Borrelia* DNA in 12% (24/205) of samples, the highest prevalence was in wild turkeys (16%; 5/31), followed by ruffed grouse (13%; 16/126) and American woodcock (3%; 1/35). All pheasants (n=13) were negative. We sequenced amplicons from all positive game birds and all were *B. burgdorferi* sensu stricto. Our results support previous work indicating that certain species of upland game birds are commonly infected with *Borrelia* species, but unlike previous studies, we did not find any relapsing fever borreliae.

Tick-borne pathogens in domestic dogs: Studies to develop spatio-temporal models to forecast the prevalence of antibodies to *Ehrlichia*, *Anaplasma*, and *Borrelia burgdorferi* in domestic dogs were continued. These models are being developed using data from several million dog testing results. We are developing these models and mapping areas with increased or decreased forecasted prevalence in dogs. These data are expected to be useful to veterinarians and physicians to determine locations where tick-borne pathogens are expected to occur. The first step in this work was to develop a large-scale Bayesian spatio-temporal binomial regression model to investigate the regional and local trends in antibody prevalence. Once developed, we tested it using about 16 million *B. burgdorferi* antibody Lyme tests performed on canine samples in the conterminous United States from January 2012 to December 2016. This analysis identified areas of increasing canine Lyme disease risk; prevalence of infection is getting worse in endemic regions and increases are also seen in non-endemic regions. This study was previously published in *Environmetrics.*

We are currently using this model to now study trends for *Borrelia* (using a larger dataset across more years), *Anaplasma* spp., and *Ehrlichia* spp. in dogs. The former two pathogens will be published together since they share the same vector, and previously we had analyzed data from 2012-2018. This year we added in data from 2019. Regional trends were not static over time, but rather increased within and beyond the borders of historically endemic regions. Increased seroprevalence was observed as far as North Carolina and North Dakota for both pathogens. Local trends were estimated to evaluate the heterogeneity of underlying changes. A large cluster of counties with increased *B. burgdorferi* seroprevalence centered around West Virginia, while a similar cluster of counties with increased *Anaplasma* spp. seroprevalence centered around Pennsylvania. In the Midwest, only a small number of counties experienced an increase in seroprevalence; instead, most counties had a decrease in seroprevalence for both pathogens. These trends will help guide veterinarians and pet owners in adopting the appropriate preventive care practices for their area and may help public health providers better understand the risk of exposure for humans. A manuscript has been submitted for publication. A similar study was done with *Ehrlichia* spp. data. Regionally, increasing seroprevalence occurred within several states throughout the central and southeastern states, including Missouri, Arkansas, Mississippi, Alabama, Virginia, North Carolina, Georgia and Texas. The underlying local trends revealed increasing seroprevalence at a finer scale. Clusters of locally increasing seroprevalence were seen from the western Appalachian region into the southern Midwest, along the Atlantic coast in New England, parts of Florida, Illinois, Wisconsin and Minnesota, and in a couple areas of the Mountain region. Clusters of locally decreasing seroprevalence were seen throughout the USA including New York and the mid-Atlantic states, Texas, the Midwest, and California. Overall, canine *Ehrlichia* spp. seroprevalence is increasing in both endemic and non-endemic areas of the USA. The findings from this study indicate that dogs across a wide area of the USA are at risk of exposure and these results should provide veterinarians and pet owners with the information they need to make informed decisions about prevention of tick exposure. This study has been published and is open access (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7106614/).

For several years we have been working on mapping and forecasting tick-borne pathogens. Although most of our data come from canine diagnostic testing, we have shown these data can be useful in predicting areas of risk for humans for those zoonotic pathogens. To better describe these tools and show the importance of animal health to human health, we published a review paper titled "Canine Vector-Borne Disease: Mapping and the Accuracy of Forecasting Using Big Data from the Veterinary Community" in the journal *Animal Health Research Reviews,* and it is available open access here: https://www.cambridge.org/core/journals/animal-health-research-reviews/article/canine-vectorborne-disease-mapping-and-the-accuracy-of-forecasting-using-big-data-from-the-veterinary-community/47413B65E74C8A313B9DF8721D85DA27/core-reader.

Natural History of Piroplasms

A project to determine the distribution and diversity of piroplasms in wildlife in North America was continued. A study to investigate the diversity of piroplasms in skunks was continued. Samples from California, Florida, Georgia, North Carolina, Pennsylvania, South Carolina, and Texas have been tested and positive samples were sequenced. Sequencing of the 18S rRNA region and cox1 gene indicates that at least two piroplasm species infect skunks. However, the *Babesia microti*-like species of skunks seems to be a species complex and thus further phylogenetic analyses of additional gene targets will be conducted to distinguish the geographic distribution and diversity of piroplasm in skunks.

We continue to be involved in investigations related to *Theileria* in wildlife, with an emphasis on *T. orientalis* (see Asian longhorned tick section above). This project began after finding *T. orientalis* Ikeda strain in a cow from Virginia. We have been investigating two primarily questions related to this parasite – what is the vector (i.e., the invasive *Haemaphysalis longicornis*) and can deer serve as a reservoir for this parasite. To stream-line testing, a restriction fragment length polymorphism (RFLP) assay was developed is being used to test for and identify *Theileria* and *Babesia* spp. in deer and other cervids with an emphasis on the detection of exotic *Theileria* spp. (e.g., *T. orientalis*) that may have been introduced with *Haemaphysalis longicornis*. The development of this assay is being written up in a manuscript for future submission. As indicated above, the WTD Theileria sp. is commonly detected but to date, , no deer have been positive for *T. orientalis.* However, a small number of *Theileria* spp. other than the WTD *Theileria* sp. have been detected and subsequence sequencing indicates they are likely variants of "*T. cervi*". Because of the high diversity of piroplasms in white-tailed deer (and other cervids, see below), we are now working on a real-time PCR that can be used for simultaneous screening and identification of the different piroplasms in cervids.

A study evaluating the presence of piroplasms in cervids from Nevada was begun. Species tested included mule deer, elk, moose, bighorn sheep, and pronghorn. To date, >250 animals have been tested. Moose were negative for any piroplasms (although only 4 were tested) and one pronghorn was positive for *Babesia odocoilei*. *Babesia duncani* (BH3 strain) was found in bighorn sheep, elk, and mule deer while *Babesia duncani (*FD strain) was found in two mule deer. A *Babesia* species that was historically found in reindeer (most similar to *Babesia* sp. RD-63) was found in elk and mule deer.

A study on piroplasms in groundhogs (*Marmota monax*) was imitated and is ongoing. To date, 50 groundhogs from various locations in Pennsylvania have been tested for piroplasms; however, none have been positive by PCR.

Feral Swine Studies

SCWDS has a long history of research and surveillance activities relating to the distribution of feral swine in the United States and the diseases associated with these populations. Currently, with funding through USDA-APHIS-Wildlife Services, SCWDS is conducting a collaborative research project with Colorado State University, and USDA-APHIS-WS to better understand the biology of parasites of importance in swine, specifically *Toxoplasma gondii* and *Trichinella* spp. The research will provide knowledge to advance the utility of the national surveillance program and targeted disease projects and allow for an assessment of food safety risk. The project involves the testing of serum samples from feral swine for antibodies to *T. gondii* and *Trichinella* spp. Sites were selected to complement and expand on two previous datasets. Laboratory processing of the samples is underway.

Dracunculus - African and North American Guinea Worms (GW)

The Guinea Worm Eradication Program's campaign to eradicate a debilitating disease in humans caused by *Dracunculus medinensis* has led to a decrease from ~3.5 million cases in 1986 to only 28 in 2018. In Chad, an unusual epidemiology has developed with dogs emerging as potential reservoirs, and it was believed that aquatic paratenic hosts, i.e., fish and/or frogs, were involved in transmission.

With funding from the Carter Center, researchers and students at SCWDS have been working with colleagues at the Carter Center and the CDC to conduct field and laboratory studies to investigate the natural history of this parasite as well as the potential role of aquatic paratenic hosts in the transmission. Although we are conducting work in Chad, Africa with *D. medinensis*, some experiments are challenging to conduct there, so we are using a local parasite, *D. insignis* from raccoons, as a model system.

We have concluded experimental studies investigating the paratenic and/or transport hosts for *D. medinensis* and *D. insignis*. Previously we found that several species of amphibians could harbor infective L3s in their tissues whereas most fish were refractory to infection. However, fish could still serve as transport hosts and infect definitive hosts if ingested while infected copepods were present in their gastrointestinal tracts. During this past year additional species were included in experimental trials, and a species susceptibility manuscript is in preparation

We also conducted a study to determine whether or not dogs can ingest copepods during a normal drinking event. This work has been published as a dispatch in the journal Scientific Reports.

We also have conducted an experiment investigating the effects of water temperature on copepod survival. In Chadian villages, water often is provided for domestic livestock and fowl. The origin of this water, however, may either be from protected bore hole wells, or from ponds that could act as a source of transmission of *D. medinensis*. The goal of our study was to evaluate the effect of temperature on copepod survival in water bowls (metal, glass, plastic) when exposed to simulated Chadian temperatures (40ºC). Initial work (consisting of 6 trials per bowl material per each time interval) was conducted using native Georgia copepods. We found a positive correlation between increasing water temperature and copepod mortality over time and metal dishes had the highest mortality rates of copepods, achieving 100% mortality at 4 hours. Plastic dishes caused the least mortality, and greater than 50% of copepods were still alive at the conclusion of 8 hr trial periods. However, this initial work was conducted with a copepod colony derived from Georgia copepods. A small set of trials was conducted using Chadian copepods, and there was no difference in survival. This work illustrates that provisioning water from animals from unprotected water sources may not be protective and that transmission of *D. medinensis* may still occur via this pathway. This work has been published in the *International Journal of Infectious Diseases*.

We have been conducting experimental trials to determine whether there is a significant difference in copepod consumption by different species of aquatic animals. We have exposed 5 species of fish and 9 species of amphibians to a constant number of copepods and allowed them to feed for 24 hrs. Remaining copepods were collected and enumerated. Data analysis shows a significant difference in numbers of copepods consumed between amphibians and fish, with fish consuming an average of 34/50 (68%) copepods, whereas amphibians consumed 08/50 (16%) in a 24 hr period. A manuscript describing this work is currently in preparation.

We have been working to develop and deploy a rapid, highly sensitive and specific field molecular assay for the detection of Guinea worm DNA in fish tissues (both muscle and entrails). Because of difficult field conditions we have been working to develop a Loop Mediated Isothermal Amplification (LAMP) assay. Prior to the decision to develop a LAMP, the plan was to survey fish guts for Guinea worm by standard parasitological methods. We conducted a survey of small fish (n=100) in Marabe, Chad during a period of time with a high number of worms emerging in dog (February-March 2019). Several suspect larvae recovered but identifications are pending molecular confirmation. However, the microscopy approach for larvae in fish entrails is challenging and an inefficient use of time in the field. Thus, the plan is to move towards a molecular method and this effort is currently underway. Over the coming year, the LAMP assay will continue to be optimized and validated in the laboratory before moving to field samples.

Although infection of dogs with Guinea worm is a major concern in Chad, the increase in the number of infected cats is also an emerging concern. We conducted a study on the foraging/scavenging/hunting behaviors of domestic cats in Chadian households. Cameras were affixed to collars of 11 household cats and deployed overnight for 17 nights in villages with a history of animal infections. Numerous events were recorded of cats eating wildlife or being fed fish guts, both possible routes of infection with Guinea worm. Data analysis is underway

In addition to domestic dogs and cats, there have been sporadic reports of Guinea worm in wildlife (wild cats, leopard, and baboons). To assess the possible risk of wildlife becoming infected in villages or being a source of local contamination of water, we initiated a study to determine the presence of wildlife near villages in Chad and assess Guinea worm transmission dynamics among wildlife and domestic dogs. Game cameras (n=8) were deployed in each of seven villages (3 with endemic Guinea worm transmission in dogs, 3 with sporadic infections, and 1 negative village). Data collection was conducted at 2-3 month intervals after camera deployment (May, August, October) with cameras to be removed from villages in October/November 2019. Preliminary analysis of photos taken by game cameras reveals a wide variety of wildlife species in close proximity to villages. Data analysis is underway, with the goal of developing an occupancy model for a variety of potential definitive host species to be completed in Spring 2021.

To date, no drug has been shown to have efficacy against preventing or treating infections with Guinea worm. There has been growing interest in flubendazole as a new drug for treatment of filarial parasites. Thus, we have been conducting trials to evaluate this new drug. One study has been on the diagnostic imaging and flubendazole treatment of Guinea worm experimentally infected ferrets. The goal of this work was to provide comprehensive diagnostic imaging (CT scan, MRI, and ultrasound) of ferrets exposed to third-stage Guinea worm larvae and then treat infected individuals with flubendazole. Ferrets were exposed during Fall 2019, and subsequently imaged. We were unable to detect infections at 8 months post exposure in any ferrets. Infected individuals (determined by appreciation of subcutaneous worms) were treated with flubendazole. Despite a small sample size, we have noted an effect on the larvae, specifically that larvae from treated ferrets did not develop correctly, displayed slower motility, and were not successful at infecting copepods. We have also been conducting a simultaneous field trial on dogs in Chad. The goal of the flubendazole trial is to conduct a randomized clinical field trial assessing the efficacy of flubendazole as a treatment or preventive for Guinea worm infection in dogs. In Summer 2019, 435 dogs were enrolled in a double-blind study. Each dog received a 1st round of treatment with either flubendazole or placebo. A subset of 80 dogs were outfitted with Iridium satellite GPS collars to track movement for 12 months of the study. A 2nd round of treatment was administered to all enrolled dogs in late October-November 2019. A 3rd round of treatment is currently underway (June-July 2020) to all remaining dogs.

Each year, we attend and present results of studies at the International Review Meeting of Guinea Worm Eradication Program Managers Meeting (occurred virtually in March). We also attended the annual International Research Agenda meeting for the Eradication of Guinea Worm. We also provide summary data and slides/talking points for both the Annual International Commission for the Certification of Dracunculiasis Eradication (ICCDE) Working Group on Animal Certification (in Switzerland or UK in June) and the intra-country updates meetings (i.e., the Chad meeting, generally in January).

SARS-CoV-2 Activities

Questions have surfaced regarding the potential risk of SARS-CoV-2 transmission from humans to North American wildlife species (reverse zoonosis). SCWDS faculty have assisted individual states and have been engaged in activities of the AFWA Fish and Wildlife Health Committee to discuss and participate in drafting guidance to for agencies on aspects of wildlife and the risk of SARS-CoV-2 infection. Scientific information to clearly define this risk is currently lacking, although ongoing and future studies will help. Although at present there is no indication that this virus can be transmitted or maintained in a North American wildlife population, transmission from humans to wildlife during routine handling or contact cannot be discounted. Risk reduction represents our first line of defense in preventing a wildlife reservoir scenario. Current strategies to mitigate this undefined risk include 1) cessation of activities that result in humans handling live animals, 2) modifying practices during such activities and/or 3) use of personal protective equipment.

SCWDS researchers recently initiated a National Science Foundation-funded project to determine the susceptibility of striped skunks and raccoons to experimental infection with a human isolate of SARS-CoV-2. In order to expand our knowledge of the host range of this virus, we are currently conducting trials to determine if two common wildlife species, raccoons and striped skunks, are susceptible to experimental infection with SARS-CoV-2. The study is underway and results are pending.

Avian Influenza Surveillance and Research

Surveillance and research continued in order to better understand the epidemiology of influenza A viruses (IAV) and avian paramyxoviruses (APMV) within North American Anseriformes (ducks and geese) and Charadriiformes (shorebirds and gulls). The goals of this work are to better define wildlife reservoirs for these viruses, gain an understanding of environmental persistence and maintenance, and provide recent field isolates for experimental and genetic studies. During this fiscal year, SCWDS conducted the following activities:

- Field studies:
    - *Northwest Minnesota:* During fall 2019 migration, 475 cloacal (CL)/oropharyngeal (OP) swabs were collected from mallards resulting in the isolation of more than 70 IAV (15% prevalence).
    - *Louisiana/Texas:* During fall migration (September 2019), collected CL/OP swabs from over 500 hunter-harvested teal (primarily BWTE) in Texas and Louisiana. Processing of this collection is 90% complete and has resulted in the isolation of 22 low pathogenicity (LP) IAV (approximate prevalence of 4.4%); subtyping of these isolates is pending.

- *Delaware Bay shorebirds*: During May 2020, traveled to Delaware Bay for collection of CL/OP and fecal swabs from 435 ruddy turnstones and gull species. Complete laboratory processing of this collection is pending, but thus far 15 LPIAV have been isolated.
- In collaboration with the USGS-Alaska Science Center (ASC) and Patuxent Wildlife Research Center (PWRC):
    - *USGS-ASC:*
        - We are carrying out another field trial to assess the environmental persistence of IAV and APMV in water under natural temperature and physical/chemical conditions for a time period consistent with an overwintering event spanning the period between fall and spring migrations. Of 206 swab samples collected from waterfowl at Izembek National Wildlife Refuge, Alaska, that were diluted in natural water, 16 were initially IAV positive and five were APMV positive by virus isolation. Persistence of these viruses in four water bodies on the refuge have been evaluated every few months. Replicate samples were tested again in December and February. In December, nine and three samples remained IAV and APMV positive, respectively; in February, seven IAV and one APMV were isolated. Due to SARS-CoV-2 restrictions, final sample collection was put on hold and will occur when collaborators gain access to remote field sites in Alaska.
        - Feces and CL/OP swabs collected from waterfowl at Izembek NWR, Alaska, in September 2019 are being screened by rrt-PCR and virus isolation for IAV.
    - *PWRC:*
        - Completed subtype-specific serologic testing (microneutralization and hemagglutination inhibition) of serum samples collected from lesser scaup in Maryland in March 2016, 2017, and 2018. Antibodies were detected against multiple HA subtypes with a high prevalence detected against H1 (39%), H6 (37%), H11 (35%), and H2 (29%).
- *Georgia/South Carolina:* In collaboration with Georgia and South Carolina DNRs and with the assistance of Jacksonville (Florida) County parks, collected more than 900 fecal samples from shorebirds (primarily ruddy turnstones; RUTU) from December 2019 through May 2020. Complete processing of these samples is pending, but thus far, 26 IAV (six in January and 20 during May migration) have been isolated. To further support this work, and to better understand the role that shorebirds using southeastern wintering and migratory stopover sites play in moving IAV into Delaware Bay, movement data collected from Lotek nanotags deployed on RUTU captured on the South Carolina coast in May 2018 and 2019 are being analyzed in collaboration with SCDNR and USFWS.
- *Seasonal and spatial trends in subtype diversity*: A multi-decade (1976-2015) collaborative study between researchers at St. Jude Children's Research Hospital, The Ohio State University and SCWDS was published in this fiscal year and revealed consistent seasonal trends related to IAV subtype diversity in North American waterfowl. During the fall, H3 and H4 IAVs consistently predominated every year. In contrast, H7 and H10 IAVs predominated every year during spring migration. An annual trend of high IAV prevalence in northern latitudes with diminishing IAV prevalence and a more diverse profile of subtypes as waterfowl migrate south was also evident in this analysis of over 77,000 samples and close to 8,500 IAV detections. The drivers of these patterns are not known but they indicate that seasonality needs to be considered when evaluating spillover risks. Results also indicate that the recovery of specific subtypes from wild bird reservoirs can be improved through seasonal targeting.

- *Identification of seasonal and annual HA antibody profiles*: Using bELISA, and microneutralization assays, work continues to better understand the role of population immunity in driving the prevalence and subtype diversity patterns of IAV in mallard populations at different temporal and spatial gradients. HA-specific antibody profiles will be determined from serum samples collected from mallards in Minnesota (fall 2017 and 2018) and gulf coast states of Arkansas and Louisiana (February 2018 and 2019).

- Laboratory Studies:
  - Collaboration with researchers at the Poultry Diagnostic and Research Center at UGA to develop reverse genetic (rg) capabilities has continued. Successful generation of five rg viruses (H3N8, H3N6, H4N6, H4N8, and H5N2) using duck-origin genes were initially characterized in vivo during an experimental study completed July 2019. Both H3 and H4 viruses replicated in experimentally infected mallards, generating humoral immune responses to challenge viruses. Duck adaptation appeared to occur in H4 rg constructs. Both duck-adapted and rg-H4 viruses were prepared for a second infection trial to test in vivo fitness, replication, and humoral immunity (planned for fall 2020). Viruses produced through the rg system will be used to better understand the potential for reassortant viruses to persist in nature and to develop better antigens for serologic tests. Successful generation and characterization of rg system for wild-bird origin viruses further improves lab capacity for characterizing avian influenza virus ecology.

West Nile Virus Surveillance

West Nile virus (WNV) continues to cause morbidity and mortality in humans, horses, and wildlife. Since 1999, WNV infection has been detected in more than 48,603 humans in the United States. During this period, over 1,955 human deaths were attributed to WNV infection. During 2019, 48 states and the District of Columbia reported WNV infections in people, birds, or mosquitoes. There were 958 human cases, including 54 fatal cases, reported during 2019 as well as WNV detections in 90 horses.

During FY2020, SCWDS continued to conduct arbovirus surveillance (including WNV) among wild birds and mosquitoes in Georgia in collaboration with the Chatham County Mosquito Control, DeKalb County Board of Health, Valdosta State University (Lowndes County), Georgia Department of Public Health, Liberty County Mosquito Control, and the City of Hinesville in support of their local arbovirus surveillance efforts: 4,778 mosquito pools and 7 dead birds submitted by Georgia county health departments and others were evaluated. West Nile virus was detected in 198 mosquito pools, Flanders virus was detected in 1 mosquito pool, a genetic variant of Flanders virus was detected in 40 mosquito pools.

Additionally, SCWDS supported the South Carolina Department of Health and Environmental Control (SCDHEC) in their arbovirus surveillance efforts. During FY2020, 514 mosquito pools and samples from 72 dead wild birds were submitted by SCDHEC for testing: WNV was detected in 2 wild dead bird samples and in 2 mosquito pools. A genetic variant of Flanders virus was detected in 5 mosquito pools and Flanders virus was detected in 1 pool.

Studies on West Nile Virus in Gamebirds

Regional ruffed grouse WNV serosurvey: During the fall of 2018, a 3-year, multi-state, West Nile virus (WNV) serologic surveillance effort in ruffed grouse (*Bonasa umbellus*) was initiated. This effort is fueled by concern over the potential impacts of WNV on ruffed grouse populations, and the objective is to gain a better understanding of the proportion of sampled grouse that have circulating antibodies specific to WNV (i.e., have survived previous WNV infection). Blood samples were collected by hunters during the 2018-2019 and 2019-2020 hunting seasons and submitted to SCWDS by individual state wildlife agencies. A SCWDS PhD student led intensive efforts to advise state cooperators as well as coordinate the testing of these samples at SCWDS. The majority of samples were collected onto Nobuto blood filter strips in the field, dried, and shipped to SCWDS for testing. Samples are tested for antibodies to WNV by virus neutralization test.

Final results from the 2018-2019 hunting season (study year 1) were previously reported to each participating state. A total of 1,287 samples were received from 13 states, 13.7% (175/1,287) of which tested positive. From the 2018-19 hunting season (study year 2), three upper Midwestern states also submitted fresh heart samples (total n=697) from hunter-harvested grouse. State-specific sample numbers included Minnesota (254), Wisconsin (235), and Michigan (208). Viruses were isolated from three heart samples, all from Wisconsin, which were each confirmed as eastern equine encephalitis virus. WNV was detected by PCR in 2 heart samples from Wisconsin, 4 heart samples from Michigan, and in 0 heart samples from Minnesota.

Submissions and testing of the blood samples collected during the second year of this project (2019-2020) are in progress. Currently this season, eight states have contributed samples (n=987): Michigan (284), Minnesota (318), New Hampshire (42), Vermont (31), Wisconsin (188), Virginia (81), North Carolina (36), and Kentucky (7). In addition, samples are anticipated from Maine, Pennsylvania, New York, and West Virginia. Three upper Midwestern states also submitted fresh heart samples (total n=779) from hunter-harvested grouse this season for assessment of active or recent WNV infection through virus isolation and polymerase chain reaction (PCR) test. State-specific sample numbers included Michigan (282), Minnesota (309), and Wisconsin (188). Viruses were isolated from four samples. Two of these isolates were from Wisconsin, and both were confirmed as eastern equine encephalitis virus. The additional two isolates were from Minnesota, and both were confirmed as Highlands J virus. WNV was not detected by PCR in any of the heart samples from Minnesota. Additional WNV PCR results are pending. Testing of all samples was delayed due to the coronavirus pandemic but has resumed and testing is currently in progress.

Wild turkey WNV serosurvey in Pennsylvania: Nobuto blood filter strips (n=194) from hunter-harvested wild turkeys (*Meleagris gallopavo)* were submitted from the Pennsylvania Game Commission for the first year of a proposed three-year, wild turkey WNV serologic surveillance study in Pennsylvania. Testing of these samples for antibodies to WNV is in progress.

WNV experimental studies: A WNV experimental infection trial has been performed in wild turkeys and northern bobwhite quail (*Colinus virginianus*), funded by the Multistate Conservation Grant Program. Experimental trials have been completed in two different age cohorts of each species. Thus far, results indicate that all WNV-inoculated wild turkeys in the younger cohort (n=12) and bobwhite quail in both the younger and older cohorts (n=10 per each age group) survived infection with no observable clinical effects. One of 12 inoculated turkeys in the older cohort exhibited hind limb weakness; further testing (i.e., immunohistochemistry) is needed to assess whether WNV may have contributed. All contact control wild turkeys and bobwhite quail survived with no clinical signs, and no virus was isolated from post-inoculation sera except for from one wild turkey in the older cohort. All turkey poults had transient, low-level viremia titers from 1-2 days post-inoculation (DPI), which generally lasted for several days (i.e., to 3-5 DPI). Less than half of inoculated bobwhite chicks (8/20; 40%) developed detectable viremia titers, which were undetectable in most (6/8) by 2 DPI. All WNV-inoculated turkey poults and bobwhite chicks developed anti-WNV antibodies by the study endpoint (i.e., 14-15 DPI). Oropharyngeal or cloacal shedding was detected in a small fraction of inoculated bobwhite chicks (4/20) and in the majority of inoculated turkey poults (20/24). Infectious WNV was detected in homogenized heart tissue from the inoculated poult that exhibited hind limb weakness and was euthanized on 6 DPI. West Nile virus was also detected in the heart, kidney, spleen, and feather of an inoculated poult that was euthanized on 2 DPI due to unrelated causes. No WNV was isolated from the heart, spleen, skeletal muscle, brain, feather, and kidney of any other poults nor from any bobwhite chicks. No gross pathology was observed in any of the birds. Preliminary histopathological examination of the tissues of the young wild turkey and bobwhite cohorts indicated that 10/12 (83%) poults and 5/10 (50%) bobwhites had mild myocarditis and 11/12 (92%) poults and 0/10 (0%) bobwhites had mild encephalitis. Histopathological examination of tissues of the older age cohorts of both species, immunohistochemical staining, follow-up laboratory testing, and data analysis are ongoing for preparation of a manuscript.

Ecology of Newcastle Disease Virus in Backyard Chickens and Peridomestic Bird Species

Our work on understanding the mechanisms by which backyard chickens transmit Newcastle disease virus to wild, peridomestic birds was finalized this year.

Newcastle disease virus (NDV), a significant pathogen of poultry, is increasingly being isolated from wild birds such as passerines. Spillover of the virus between chickens and wild bird species is increasingly documented, yet the ecological reasons behind spillover events remains unclear. We examined how lentogenic NDV transmits from infectious chickens to passerines and near passerines using an experimental administration performed under natural field conditions. Using a live NDV vaccine, the LaSota vaccine virus, we inoculated chickens living under two conditions at different sites in Athens, Georgia. The flock at Site S was maintained in a coop both day and night, whereas the flock at Site L was maintained as a flock of free-ranging chickens during the day. As a control, we also surveyed the wild birds at an additional site without backyard chickens. At the two sites in which the chickens were inoculated, we identified 13 wild bird species (of the 46 species captured) that had seroconverted and were identified as ‘high’ risk for NDV transmission from backyard chickens. Across all sites combined, these 13 high-risk species had 197 out of 351 captures (56%) near the backyard chicken coops. In addition, chipping sparrows (*Spizella passerina*), northern cardinals (*Cardinalis cardinalis*), and tufted titmice (*Baeolophus bicolor*) had the highest antibody responses across all three sites. Our results provide evidence that NDV spillover between passerines and backyard chickens occurs, and that inhibiting spillover events may also be crucial to the management of NDV outbreaks in backyard chickens, specifically those that share habitat with wild birds.

At each of the aforementioned sites, we identified the species of wild birds that: a) shared habitat with the chickens, b) the frequency of detection, and c) encountered the chicken coops. For the species that encountered chicken coops, we also measured the contact rate with which they did so. We identified 14 wild bird species of the 72 species recorded that entered the coops to consume supplemental feed and were considered ‘high’ risk for pathogen transmission. Across all sites combined, these 14 high- risk species had 589 out of 1,574 total contacts with the backyard chicken coops. Chipping sparrows, northern cardinals, and tufted titmice had the highest contact rates across all three sites. We found that site species richness was more predictive of wild bird contacts with backyard chickens than whether chickens free-roamed or were cooped. Our results provide evidence that contact between wild birds and backyard chickens is a common occurrence, which has crucial implications for pathogens shared among these species.

We also performed a review of the pathogen transmission at the backyard chicken-wild bird interface for a review manuscript. We reviewed the literature on spillover events of pathogens historically associated with poultry into wild birds. We also reviewed the reasons for biosecurity failures in backyard flocks that lead to those spillover events and provide recommendations for current and future backyard flock owners.

Gamebird Health Surveys

Oklahoma Northern Bobwhite Health Survey

Oklahoma Department of Wildlife Conservation (ODWC) and SCWDS are conducting a health survey of the Northern bobwhite quail (*Colinus virginianus*) in western Oklahoma. The overall goal of the project is to create a baseline health data set of the bobwhite population across western Oklahoma. We are sampling quail at 10 wildlife management areas over a two year period to accomplish the following objectives: (1) evaluate the body condition of the quail and evaluate for evidence of disease or/and abnormalities during postmortem examination, (2) collect and identify any external or internal parasites, (3) screen for blood parasites using molecular assays, (4) conduct histological examination on tissues from a subset of quail, (5) test for West Nile Virus exposure/infection, and (6) screen a subset of quail for exposure to neonicotinoids.

This past year (2019) was the first full year of the project. Bobwhites (n=96) were collected during the late summer and early fall of 2019, and shipped to SCWDS for necropsy examination. Generally, the quail were in good to excellent nutritional condition (muscle mass, visceral fat, subcutaneous fat). Adult birds (n=36) had an average body weight of 181 grams. For birds collected during the August period, ectoparasites were found on 77% of birds, intramuscular parasites were observed in 12% of birds, gastrointestinal parasites were observed in 49% of birds, and 11% of birds had eye worm (*Oxyspirura petrowi*) infections. For the October collection, ectoparasites were found on 100% of birds, intramuscular parasites were observed in 11% of birds, gastrointestinal parasites were observed in 72% birds, and 19% of birds had eye worm infections. All blood samples tested negative for blood parasites, including *Haemaproteus*, *Leucocytozoon*, and *Plasmodium,* by PCR. Tissues from all birds were tested for West Nile virus by virus isolation; all were negative. Histology of selected tissues, PCR detection of *Atoxoplasma*, *Sarcocystis*, *Mycoplasma*, and screening for neonictinoids are underway. The next field season for this project will begin in August 2020.

Wild Turkey Pathogen Survey in North Carolina

SCWDS is collaborating on a wild turkey project with North Carolina Wildlife Resources Commission (NCWRC) and North Carolina State University (NCSU). Researchers at NCSU are conducting a multi-year telemetry study of wild turkeys to better understand multiple aspects of their biology and ecology to better inform management strategies. The work is occurring in three distinct physiographic regions (Coastal Plain, Piedmont, Blue Ridge) within North Carolina. SCWDS is coordinating diagnostic testing of samples collected in the field, including testing for lymphoproliferative disease virus, reticuloendotheliosis virus, *Mycoplasma* spp., turkey coronavirus, *Salmonella*, *Borrelia* spp, hemoparasites, WNV antibodies, AIV antibodies, APMV antibodies, and *Toxoplasma gondii* antibodies. January to March 2020, turkey trapping and sampling occurred and samples were received from each region: Coastal Plain, 32 birds; Piedmont, 83 birds, and Blue Ridge, 81 birds. Laboratory processing of samples has been delayed because of COVID-19 restrictions but has been initiated and results are pending. Additional sampling will occur in 2021 and 2022.

White Ibis Health

We continue the American white ibis project, in which we are studying the role of human resource provisioning in urban areas on pathogen dynamics in free-living adult and juvenile white ibis, and captive nestling white ibis. We have finalized most of our work, and we have published or submitted four manuscripts. New to this year was the beginning of two graduate student projects: 1) monitoring the productivity and pathogen dynamics of a white ibis rookery in an urban setting in Palm Beach County and, 2) investigating the antimicrobial resistance of white ibis and European storks across an urbanization gradient. The second project was postponed due to the global pandemic and travel restrictions.

Ibis only began breeding in urban habitats very recently. In fact, only a single rookery—adjacent to the Palm Beach County landfill—was embedded in urban Palm Beach County until very recently. In the last five years, ibis have begun nesting in two urban rookeries, one of which is readily accessible to researchers. Similar highly urbanized species have shown higher productivity in urban habitats. We began data collection in April 2020 at this urban rookery (monitored 64 nests). We collected growth and pathogen data from 36 nestlings. We will compare the productivity of this colony to natural rookeries. So far, we have found that the urban rookery was productive, with 77% nests monitored producing nestlings and 66% successfully fledged, but we have not yet compared this to the data from natural rookeries for the 2020 reproductive season. We will also compare the exposure of urban nestlings to pathogens such as *Salmonella* and avian influenza viruses.

Snake Fungal Disease

Surveillance of clinical case submissions continued for *Ophidiomyces ophiodiicola* (*Oo*), the fungal agent that causes ophidiomycosis, also known as snake fungal disease. Three carcasses of the following species were examined: scarlet kingsnake (*Lampropeltis elapsoides*), eastern indigo snake (*Drymarchon couperi*), and eastern ratsnake (*Pantherophis alleghaniensis*). Samples came from Alabama, Florida, and Georgia, respectively. Snake fungal disease was diagnosed in the scarlet kingsnake and the eastern indigo snake by confirmation of histological lesions and the presence of *Oo* by PCR

Bsal and other emergent pathogens of salamanders in Costa Rica

We finalized a graduate student's thesis to explore relevant amphibian pathogens in salamanders in Costa Rica. We did this by conducting a surveillance project for *Batrachochytrium salamandrivorans* (*Bsal)*, *Batrachochytrium dendrobatidis* (*Bd*), and ranavirus and by developing a risk-assessment model to examine regions with high-risk of *Bsal* introduction. Both manuscripts are in preparation.

Batrachochytrium salamandrivorans is an emergent fungal pathogen that has caused significant population declines in Northern European salamander populations after being introduced from East Asia, presumably via the pet trade. *Bsal* has not yet been detected in the Americas, though surveillance for the pathogen has been limited to the United States, Canada, and Mexico. In this study, we initiated surveillance for *Bsal* in Costa Rica, a country whose multi-billion-dollar ecotourism industry and mountainous habitats, ecologically suitable for *Bsal*, make it a possible point of introduction for the pathogen into the Americas. We hypothesized that if *Bsal* was present it would most likely be detected in areas with high human visitation. Because of the history of *Bd* in Costa Rica, we hypothesized *Bd* would be detected at least at low prevalences. We hypothesized that *Ranavirus* would not be detected because our sample sites were not ecologically ideal for the virus. Between December 2018 and December 2019, we sampled 57 salamanders (47 *Bolitoglossa pesrubra* and 10 *B. cerroensis*) at five sites in Cerro de la Muerte and 43 salamanders (39 *B. subpalmata* and 4 *Nototriton abscondens*) in Parque Nacional Volcan Poas. All salamanders were negative for all three pathogens.

Some surveillance for *Bsal* has been conducted in Costa Rica, but, in order to inform continued targeted surveillance, we developed an Ecological Niche Model (ENM) using MAXENT in ArcGIS to identify areas ecologically suitable for *Bsal* in Costa Rica. We sought to identify areas at high risk of *Bsal* introduction by finding where areas of ecological suitability overlap with high human visitation and high salamander biodiversity. We predicted suitability would be highest in Costa Rica's Cordillera Central and Cordillera Talamanca, that 30 salamander species would live in areas suitable for *Bsal*, and that protected areas in the aforementioned mountain ranges would be points of high suitability and visitation. We found that ~23% of Costa Rica's landmass is suitable for *Bsal*, that 27 salamander species, including 4 endangered species, live in areas suitable for *Bsal*, and identified eight areas in at which suitability intersected with high biodiversity and/or human visitation. Many of these locations were within the Cordillera Central and Cordillera Talamanca and we encourage pathogen surveillance and research in these areas.

White-Nose Syndrome

SCWDS continued testing for white-nose syndrome (WNS) and its causative agent, *Pseudogymnoascus destructans* (*Pd*), in bats from the Southeast. We examined 39 samples representing 38 carcasses for WNS and/or the presence of *Pd*. Samples were submitted from Arkansas, Florida, Georgia, Kansas, Missouri, Nebraska, North Carolina, Pennsylvania, South Carolina, and West Virginia. No suspect or confirmed infections with *Pd* were detected. Species tested included big brown bat (*Eptesicus fuscus*), Brazilian free-tailed bat (*Tadarida brasiliensis*), eastern red bat (*Lasiurus borealis*), evening bat (*Nycticeius humeralis*), Florida bonneted bat (*Eumops floridanus*), Indiana bat (*Myotis sodalis*), little brown bat (*Myotis lucifugus*), silver-haired bat (*Lasiurus noctivagans*), and southeastern myotis (*Myotis austroriparius*).

Animal Health Research Center

Dr. Mead continues to serve as Science Director of the Animal Health Research Center (AHRC), the College of Veterinary Medicine's high security, high containment, research facility. In this role, Dr. Mead serves as the Division of Select Agents and Toxins registered user and provides administrative oversight and review of research projects in the AHRC. Additionally, he is responsible for ensuring that the facility and operations are in compliance with applicable laws, regulations, and guidelines pertinent to biocontainment and infectious disease research programs.

Brucella Vaccine Competition

In January 2018, researchers in the College of Veterinary Medicine at the University of Georgia were awarded a phase 1 prize of $100,000 for their application to the *Brucella* vaccine competition sponsored by AgResults and managed by the Global Alliance in Livestock Veterinary Medicines. Dr. Mead is one of the investigators on the project. The goal is to develop a safe and efficacious vaccine for ovine and caprine brucellosis caused by *Brucella melitensis*. The UGA team is using a new vaccine platform to deliver *Brucella* antigens that provide complete, long-lasting, and cross-species immunity against pathogenic *Brucella* bacteria. Vaccine safety and efficacy trials in goats were conducted in the last year. The safety trial was conducted to determine if the vaccine would cause abortion in pregnant goats as is seen with the *B. melitensis* Rev1 vaccine. A group of 9 pregnant goats received the experimental vaccine, and another group of 9 pregnant goats received a sham vaccine. Goats were held on pasture and allowed to go to term. A single abortion occurred in the vaccine group, but it was determined that the doe had a respiratory infection and high fever and this was the cause of the abortion. No differences were found between groups. An efficacy study was conducted by infecting vaccinated and sham vaccinated goats with wild-type *Brucella melitensis*. Goats were challenged via a route that causes disseminated infection (inoculation of conjunctival sac). Goats were held for 20 days then humanely euthanized and necropsied. Bacterial counts were conducted from select tissues and organs. Bacterial counts in vaccinated goats were significantly lower than bacterial counts from sham vaccinated goats. Pending additional funding, a second efficacy study in pregnant goats using a challenge route more in line with natural infection is being planned.

National Fish and Wildlife Health Initiative

SCWDS has been involved in the National Fish and Wildlife Health Initiative (NFWHI) since it was first discussed in the spring of 2005. John Fischer served as vice chair of the AFWA Working Group drafting the NFWHI. Guiding principles for the initiative were endorsed by AFWA in September 2005, an AFWA resolution was passed in support of development and implementation of the initiative, and a supporting resolution was passed by the United States Animal Health Association (USAHA) in November 2005. An outline of the initiative, containing six principle strategies, was endorsed by AFWA at its business meeting in March 2007 and a Steering Committee, comprising appropriate state, federal, and university personnel, was appointed to develop an implementation plan.

The two over-arching goals of the Initiative are to:

- Facilitate establishment and enhancement of state, federal, and territorial fish and wildlife management agency capability to effectively address health issues involving free-ranging fish and wildlife.
- Minimize the negative impacts of health issues affecting free-ranging fish and wildlife through surveillance, management, and research.

In March 2013, the AFWA Fish and Wildlife Health Committee elected to reignite the NFWHI that had been put into stasis in 2011 because of state and federal budget difficulties. John Fischer served as vice chair of the NFWHI Steering Committee and participated in meetings of this group in September 2015, August 2016, March and September 2017, and March 2018. During a July 2019 meeting organized by AFWA and attended by Paul Johansen and David Stallknecht a decision was made to move on this proposal. In February 2020, SCWDS hosted the Fish and Wildlife Health Strategic Planning Retreat, and efforts to advance the proposal are ongoing.

Informational Activities

Since July 1, 2019, SCWDS personnel have provided assistance with informational items related to wildlife health on numerous occasions and in many forms. Four issues of the quarterly newsletter, the SCWDS BRIEFS, were prepared and distributed in hard-copy format to more than 1,600 individuals worldwide and via electronic copy to more than 700 individuals and organizations. Many newsletter recipients now use online access to download and print copies rather than receive a paper copy via mail. Over the next year, SCWDS will work to modernize the newsletter and move towards an online format. SCWDS staff members attended at least 65 meetings to make presentations on wildlife health issues. Some presentations were at scientific sessions, and others were in response to requests for information. In addition, SCWDS provided consultation or information in response to more than 1000 inquiries during the year.

SCWDS has continued to update and modify the web page (https://vet.uga.edu/scwds). The University of Georgia is in the process of changing the College of Veterinary Medicine's website and significant improvements to the SCWDS web page will occur over the coming year. Currently, the website format has changed, but it contains the same information that should be helpful to individuals and agencies seeking information regarding SCWDS. The website includes: 1) a history and description of SCWDS; 2) information on diagnostic services including a necropsy submission form that can be downloaded; 3) a description of SCWDS veterinary externship and graduate degree programs; 4) information and order forms for the *Field Manual of Wildlife Diseases in the Southeastern United States;* 5) a SCWDS personnel roster including e-mail addresses; 6) a list of recent SCWDS publications; 7) current and archived copies of our newsletter, the SCWDS BRIEFS, (full text now searchable at the University of Nebraska's DigitalCommons@UNL website (http://digitalcommons.unl.edu/secwds/); 8) a topic index of the SCWDS BRIEFS with information on a variety of wildlife health topics; 9) electronic version of the updated hemorrhagic disease brochure; 10) electronic versions of various big game density and distribution maps prepared by SCWDS; 11) information on the Southeastern Wildlife Health Development Fund; 12) a list of SCWDS Steering Committee Members; and 13) links to member state wildlife agency home pages.

ACADEMIC AFFAIRS

SCWDS Teaching Program

SCWDS has a strong educational program and is fortunate to be able to select highly qualified and motivated students for graduate training with an emphasis on wildlife population health. The number of assistantships is limited by funding availability. Graduate degrees, MS and PhD, are available in The University of Georgia's (UGA) College of Veterinary Medicine or the D.B. Warnell School of Forestry and Natural Resources, depending upon the student's interest. Within the College of Veterinary Medicine, SCWDS graduate students can enter the Comparative Biomedical Sciences (CBS) graduate program and complete customizable MS and PhD programs that emphasize interdisciplinary research and translational science. Students can tailor their program of study to their particular needs, interests, and goals.

SCWDS has continued to provide educational opportunities for veterinary students at UGA. Drs. Yabsley and Hernandez taught a Wildlife Health Elective (POPH 5201). Dr. Yabsley, with faculty from the Department of Infectious Diseases, co-taught Veterinary Helminthology and Ectoparasitology. Dr. Yabsley taught a 1-cr seminar on wildlife diseases for graduate students in Fall and Spring semesters (POPH 8580). Dr. Mead taught a one credit veterinary elective, Public Health (POPH 5180).

Dr. Yabsley offered a FYOS course to freshman on wildlife diseases and why they are important. Dr. Yablsey taught Principles and Management of Wildlife Diseases (WILD 5100/7100). Dr. Hernandez was co-instructor for Ornithology (WILD 4060/6060) for which she taught half of the lectures and all the labs. Dr. Hernandez also taught Wildlife Disease Ecology: Investigation and Management (WILD 8500) to graduate students and a FYOS course on ornithology. In the Fall 2019, Dr. Hernandez taught a new FYOS course on One Health which is also being offered in Fall 2020. Drs. Yabsley and Hernandez also had students enrolled in various undergraduate research courses (e.g., HONS 4960H, 4970H, and 4980H, and FANR 4600).

Wildlife Health Elective

SCWDS offers a block rotation in wildlife population health for senior veterinary students at the UGA College of Veterinary Medicine and externships for senior students from other veterinary colleges in the United States and other countries. These rotations enable students to work with wildlife health professionals in a variety of capacities. Students generally are involved in the diagnostic service and conduct necropsies on wildlife submitted to SCWDS by partner agencies. Students also assist with local and regional field projects and occasionally with laboratory research. All students also complete a written assignment and an oral presentation on relevant wildlife health topics. Drs. Nemeth, Ruder, Kunkel, Dugovich, Weyna and Radisic, as well as others at SCWDS, provided instruction and guidance for eight veterinary students from Michigan State University, University of California-Davis, University of Georgia, Lincoln Memorial

University, and Virginia-Maryland College of Veterinary Medicine. Unfortunately, numerous cancellations occurred due to the COVID-19 pandemic but students will again be allowed to participate beginning August 2020.

Graduate Studies

SCWDS offers graduate degree programs focusing on wildlife health-related research in the College of Veterinary Medicine (CVM) and the D.B. Warnell School of Forestry and Natural Resources (WSFNR). This graduate instruction includes close collaboration with faculty strictly affiliated with SCWDS and faculty with wildlife health expertise within other academic departments in the CVM. During FY2020, over forty graduate students were affiliated with SCWDS faculty:

Dr. Sonia M. Hernandez served as Major Professor for the following PhD students: Andrea Ayala (CVM; graduation August 2020), Jorge Rojas (Warnell; expected graduation December 2023), and Sebastian Ortiz (Warnell; graduation expected Dec 2020); and the following MS students: Henry Adams (Warnell; graduation August 2020), Raquel Francisco (expected graduation August 2021), Julia Silva (expected graduation August 2021) and Katherine Christie (expected graduation December 2021). She also served on the Graduate Committees of the following students: Sam Merker (PhD; Warnell); James Leaphart (PhD; Warnell/SREL); Cali Wilson (PhD; Ecology); Anna Willoughby (PhD; Ecology); Accepted graduate student that will begin in Fall 2020: Matthew Tatz (Warnell; MS).

Dr. Daniel Mead served as co-Major Professor for one UGA DVM/MPH student (Rochelle Medford - Epidemiology, College of Public Health; CPH). Additionally, Dr. Mead served as the co-Major Professor of one UGA PhD Student (Kayla Guinn Adcock - Biomedical and Health Sciences, CVM) and served on the Graduate Committees of four UGA PhD students (Jeremy Dyke - Department of Infectious Diseases, CVM; Sally Boyd - Biochemistry and Molecular Biology; Blanka Tesla - Department of Infectious Diseases, CVM; Melanie Kunkel, Biomedical and Health Sciences, CVM).

Dr. Nicole Nemeth served as Major or Co-advisor of PhD students Melanie Kunkel (Department of Population Health, CVM, UGA), Kayla Guinn Adcock (Department of Infectious Disease, CVM, UGA), Chloe Goodwin and Alisia Weyna (both Department of Pathology, CVM, UGA), and Samantha Allen (Department of Pathobiology, Ontario Veterinary College, University of Guelph), and DVSc student Christina McKenzie, (all Department of Pathobiology, Ontario Veterinary College, University of Guelph). She also served on the PhD Committee of Justin Stillwell, Shannon Kirejczyk, and Alicia O'Toole (Department of Pathology, CVM, UGA), and MSc Committees of Seth Wyckoff (Department of Population Health, CVM, UGA).

Dr. Rebecca Poulson served on the Graduate Committee of CVM student Jo Anne Crum Bradley (Department of Population Health).

Dr. Mark Ruder served on the PhD Committees of Kevin Niedringhaus, Melanie Kunkel, and Alec Thompson within the Comparative Biomedical Sciences program in the College of Veterinary Medicine. He also served on the MS Committee of Seth Wyckoff and Seth White (WSFNR) and on the PhD committee of Samantha Allen (Department of Pathobiology, University of Guelph).

Dr. David Stallknecht served as Major Professor for one PhD student in the CVM: Jo Anne Crum Bradley (Department of Population Health). He also served on the Graduate Committees of Joseph Hicks (Infectious Diseases), Silvia Carnaccini (Pathology), and Brianna Williams (WSFNR).

Dr. Michael Yabsley served as Major Professor for five MS students (Lauren Lacefield (graduated), Seth Wyckoff, Seth White, Erin Box, and Ryan Grunert) and two PhD students (Brianna Williams and Alec Thompson). He was also a co-advisor to one MVetScie in

Conservation Medicine at University of Edinburg. He also mentored two post-doctoral research associates (Jenna Gettings and Christopher Cleveland) and a PREP student (Kristen Dominguez). Dr. Yabsley served on the Graduate Committees of Pooja Gupta (graduated), Roxanne Charles, Kimberly Neil, Sebastian Ortiz, Wayne Sylvester, Henry Adams (graduated), Andrea Ayala (graduated), Julia Frederick, and Emma Browning. Dr. Yabsley also served as Faculty Research Mentor for several undergraduate students and a Young Scholars Student (high school program).

FUNDING CONTRIBUTIONS BY NON-STATE SCWDS COOPERATORS AND OTHER SOURCES

Emerging Animal Disease in the United States: Surveillance and Response

(USDA-APHIS-Veterinary Services Cooperative Agreement FAIN AP20VSCEAH00C041)

Major Investigators: Mark G. Ruder, Stacey Vigil, Michael J. Yabsley, and Nicole M. Nemeth

April 1, 2020 - March 31, 2021

The Cooperative Agreement is between the Southeastern Cooperative Wildlife Disease Study and Veterinary Services, Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture.

The objectives of the Cooperative Agreement are as follows:

- To conduct surveys for cattle fever ticks on wildlife in Texas.
- To conduct surveys for ticks, including *Haemaphysalis longicornis*, in the eastern US.
- To conduct surveys for *Culicoides* spp. in the southeastern US.
- To conduct orbivirus surveillance in wild ruminants in the US.
- To provide presentations, prepare manuscripts for publication, and provide consultation.

Research and Training to Support the National Feral Swine Damage Management Program

(USDA-APHIS-Wildlife Services, Cooperative Agreement #19-7100-0361-CA; AP19WSHQ0000C018)

Major Investigators: Michael J. Yabsley and Nicole M. Nemeth

September 11, 2019 - September 10, 2021

This cooperative agreement between USDA-APHIS-WS National Feral Swine Damage Management Program and the Southeastern Cooperative Wildlife Disease Study (University of Georgia) is intended to facilitate cooperation between our agencies in order to meet specific research/surveillance needs regarding *Toxoplasma gondii* and *Trichinella* spp. in feral swine.

The objectives of this project are:

The agreement is to support The University of Georgia to conduct research to better understand the biology of parasites of importance in swine, specifically *Toxoplasma gondii* and *Trichinella* spp. These data will provide knowledge to advance the utility of the national surveillance program and targeted disease projects and allow for an assessment of food safety risk.

- Design and implement surveillance for antibodies to *T. gondii* and *Trichinella* spp. in feral swine in the United States, analyze findings, and publish manuscripts summarizing the data.

All primary collaborators involved in obtaining data in previous datasets as well as the currently involved individuals would be offered co-authorship.

- Test samples for active infection with *T. gondii* and *Trichinella* spp. on a limited number of available fresh tissue samples
- Compare general trends in prevalence between new data on antibody prevalence for *T. gondii* and *Trichinella* spp. with previously published data from 2006-2010 (Hill et al., 2014).
- Submit an annual summary report of all activities
- Submit a final report on all activities, if all activities are completed within the first year then the annual report will serve as the final report.

NIAID Centers of Excellence for Influenza Research and Surveillance

(St. Jude Children's Research Hospital, Inc, Contract #HHSN272201400006L, Sub-contract #111978011-7615375)

Major Investigators: David E. Stallknecht, Daniel G. Mead, Elizabeth W. Howerth, and Monique Franca

April 1, 2019 - March 31, 2021

The objectives of this project are:

- To develop improved strategies for field detection of AIV and the identification of free-living avian influenza reservoirs.
- To develop a clearer understanding of AIV transmissibility and maintenance in wildlife populations.

Influenza A Viruses in Wild Birds Inhabiting the Atlantic Flyway

(USGS Alaska Science Center G19AC00391).

Major Investigators: Rebecca Poulson

September 15, 2019 - September 14, 2021

The objectives of this project are:

- To assess the prevalence of IAVs, including HP clade 2.3.4.4 viruses introduced in North America in November 2014, in dabbling and diving ducks sampled in the Atlantic Flyway by collaborators at the USGS Patuxent Wildlife Research Center (PWRC).
- To assess the genetic relationship among IAVs isolated from dabbling and diving ducks in the Atlantic Flyway with those isolated from wild and domestic birds elsewhere in North America.
- To disseminate information on the prevalence and genetic characterization of IAVS in wild birds sampled in the Atlantic Flyway to stakeholders, including natural resource managers and poultry producers, through the publication of two or more products in peer-reviewed scientific journal outlets.

Exploring the Potential for In Utero Transmission of CWD Prions in White-tailed Deer

(Association Fish and Wildlife Agencies GR-FP00018658, Sponsor Award F20AP00172).

Major Investigators: Mark Ruder

January 1, 2020 - February 28, 2021

The objectives of this project are:

- To determine if CWD prions are vertically transmitted from naturally infected white-tailed deer to fawns *in utero*. We hypothesize that efficient *in utero* transmission of PrP^{CWD} occurs in white-tailed deer. Based on existing data from naturally infected elk (80% transmission to fetuses) and experimentally infected muntjac (100% transmission to fetuses), our target of five, CWD-positive, pregnant does will be sufficient to document vertical transmission if it occurs commonly in WTD.
- *Sub-objective 1*: Examine the association between vertical PrP^{CWD} transmission and the distribution of PrP^{CWD} in doe tissues, excreta and bodily fluids. Meeting this objective will require testing of multiple tissues and samples from infected does and will provide information that may be valuable in predicting the potential for vertical transmission and the potential for direct contact transmission between does and fawns.
- *Sub-objective 2*: Compare the observed PrP^{CWD} prevalence using traditional tests (i.e., ELISA and IHC) vs. amplification assays (e.g., sPMCA).

Susceptibility of North American Game Birds to West Nile Virus
(USFWS Cooperative Agreement #F19AP00089)
Major Investigators: Nicole Nemeth, Mark Ruder, Daniel Mead, Rebecca Poulson, and
David Stallknecht

January 1, 2019 - December 31, 2020

The goal of this research is to provide an initial assessment of the pathogenic potential of West Nile virus to negatively affect wild turkey and northern bobwhite populations. Data generated from this research will provide technical diagnostic guidance and justification for more complex field studies to better understand and document actual population effects in these species.

The objectives of this project are:

- To evaluate the susceptibility of wild turkeys and northern bobwhites to WNV morbidity and mortality.
- To determine WNV-induced viremia and oropharyngeal and cloacal shedding profiles as well as viral titers in select tissues.
- To compare anti-WNV antibody titers in serum vs. Nobuto filter paper strip-eluted samples in experimentally infected wild turkeys and northern bobwhites.
- To communicate research results and relevant applications to state and federal game bird biologists and managers and other interested parties.

Diagnostic, Field, and Training Assistance for Avian Health and Disease Monitoring
(USFWS Cooperative Agreement #F15PX01848)
Major Investigators: Mark G. Ruder and Nicole M. Nemeth

August 1, 2019 - July 31, 2020

The goal of this program is to establish an avian health baseline, identify existing and emerging avian health and disease risks, ensure disease preparedness and prevention, and develop, guide, and implement appropriate and effective management actions.

The objectives of this project are:

- To perform on an as-needed basis in response to mortality events or project needs gross and microscopic pathology, radiography, microbiology, parasitology, toxicology.

- To identify and investigate diseases impacting avian populations; collection and processing of biological specimens for the USFWS Avian Health and Disease Program regional projects; field and laboratory support to Refuges, USFWS field stations, and Avian Health and Disease Program Regional Coordinators.
- To provide avian health and disease outreach activities to Refuges, USFWS field stations, assistance in organizing and presenting regional and national training courses, provision of educational materials for course, fact sheets, and web communications.

USDI Wildlife Disease Problems

(USGS-Piedmont CESU, Cooperative Agreement #G18AC00319)

Major Investigators: Mark G. Ruder

September 12, 2018 - August 31, 2023

The objectives of this project are:

- To contribute to the online Wildlife Health Information Sharing Partnership-Event Reporting System (WHISPers). Diagnostic case reports will be provided on a quarterly basis (January, April, July, October) to USGS Ecosystems Mission Area and USGS's National Wildlife Health Center (NWHC) in an Excel format using the template provided by NWHC. This information will be included in WHISPers, and SCWDS will be acknowledged to provide online visibility of the partnership between SCWDS and DOI/USGS. Reports will include but not be limited to:
    a. Wildlife submissions from DOI agencies (e.g., deer herd health evaluations on National Wildlife Refuges or National Parks)
    b. Federal species of interest (migratory birds, threatened and endangered species)
    c. Bats (including WNS cases)
    d. Amphibians (including chytrid fungi cases)
    e. Reptiles (including snake fungal disease)
    f. Reports of disease in invasive species and/or the detection of invasive or non-native animal pathogens
- To partner with USGS on national disease diagnostics, surveillance, and research for avian influenza and white-nose syndrome (i.e., national WNS Diagnostics Working Group led by USGS).
- To disseminate wildlife disease information through the quarterly newsletter, The SCWDS BRIEFS, which will be mailed and sent electronically to subscribers.

Randomized Clinical Trial Evaluating the Effect of Flubendazole as Treatment for Infection with Guinea Worm (*Dracunculus medinensis*) in Domestic Dogs, Chad, Africa.

(The Carter Center)

Major Investigator: Christopher Cleveland and Michael Yabsley

July 1, 2018 - December 31, 2020

The project aims to test the efficacy of flubendazole (FBZ) subcutaneous injection as a preventative and/or treatment method Guinea worm (GW-*Dracunculus medinensis*) infection in domestic dogs.

The objectives of this project are to enroll 600 dogs on a double blind randomized trial assessing the efficacy of Flubendazole on treatment of Guinea worm. After the first enrollment period (May-June 2019), 435 dogs were successfully enrolled, microchipped, treated, provided with a rabies vaccination, and had biological and ectoparasite samples collected. In addition, 80 GPS collars were deployed on a random subset of animals to provide movement data. A second round of treatment will begin late October 2019 on the same individual dogs enrolled initially.

Conducting Epidemiological Modeling Using the Australian Animal Disease Spread Model (AADIS)

(USDA-APHIS-Veterinary Services Cooperative Agreement #AP18VSCEAH00C018)

Major Investigators: Mark G. Ruder

September 24, 2018 - September 22, 2020

The objectives of this project are:

- To populate a regional model of AADIS with U.S. data to support the within herd and between herd spread of vector-borne diseases. Through parameter development for a U.S. regional model in AADIS, critical data requirements and gaps will be identified to inform field data collection efforts. Additionally, through collaboration, CEAH analysts will develop expertise to support the use of AADIS to assist with animal health decision making.
- The modification of the AADIS FMD model framework to support modeling the U.S. livestock herd (~2 million) agents. AADIS will be configured to simulate the spread of disease in the U.S. national livestock herd, and the stability, reliability and performance of the model will be evaluated.

Vector-Borne Disease Surveillance and Mosquito Diagnostic Support

(Chatham County Board of Commissions)

Major Investigator: Daniel G. Mead

July 1, 2018 - June 30, 2023

The goal of this project is to provide diagnostic support for mosquito pool testing for arbovirus surveillance in Chatham County.

Vector-Borne Disease Surveillance and Mosquito Diagnostic Support

(South Carolina Department of Health and Environmental Control Contract# 4400019054, PO 5400015573))

Major Investigator: Daniel G. Mead

July 5, 2018 - July 1, 2020

The goal of this project is to provide diagnostic support for mosquito pool testing for arbovirus surveillance in South Carolina.

Vector-Borne Disease Surveillance and Mosquito Diagnostic Support

(DeKalb County Board of Health #19-0823-C0001-00)

Major Investigator: Daniel G. Mead

July 1, 2018 - July 1, 2020

The goal of this project is to provide diagnostic support for mosquito pool testing for arbovirus surveillance in DeKalb County.

Comparison of Different Surveillance Methods for Modeling Dispersal of Ticks by Wildlife Hosts

(USDA-APHIS-Veterinary Services, Cooperative Agreement #AP17VSCEAH00C015

Major Investigators: Michael J. Yabsley

September 17, 2017 - September 14, 2019

The goal of this agreement was to create datasets for use in the development of APHIS's predictive tick species distribution models.

The objectives of this project were:

- To collect ticks using different surveillance methods from habitats in the Piedmont (Georgia) and Lower Coastal Plan (Georgia) physiographic regions of the United States.
- To identify tick species and associated tick-borne disease pathogens collected in these regions. Time and resources dedicated to each collection method will be recorded to economically compare tick collection methods.

Feral Swine Diseases Information and Training

(USDA-APHIS-Wildlife Services, Cooperative Agreement #AP18WSHQ0000C012)

Major Investigators: Nicole M. Nemeth and Mark G. Ruder

September 11, 2018 - September 10, 2019

The goal of this program was to provide training to control and minimize the damage from feral swine.

The objectives of this project were:

- To develop and enhance training opportunities for WS personnel relative to disease issues in feral swine.
- To provide WS personnel with one formal training and informational seminar annually to familiarize WS personnel with selected diseases of feral swine. Presentations will include information on causative agent, clinical signs, lesions, hosts, transmission, wildlife management significance, public health significance, and agricultural significance.
- To develop an informational brochure on parasites for use by WS personnel and for distribution by WS personnel.

Experimental Studies on Dogs as Hosts for Guinea Worm

(The Carter Center)

Major Investigators: Michael J. Yabsley

May 1, 2017 - December 31, 2019

The objectives of this project were:

- To determine if domestic dogs ingest copepods when drinking water. An experiment to determine this will be conducted using lab-raised, uninfected copepods and 12 purpose-bred dogs.
- To evaluate the efficacy of Advantage® (Bayer) for the treatment and prevention of *Dracunculus* infections in dogs and ferrets.
- To generate serum samples for the detection of antibodies to *Dracunculus* in dogs.
- To establish *D. medinensis* in domestic ferrets to be used in future studies.

Spatial and Temporal Aspects of the Distribution of Lyme Disease
(Companion Animal Parasite Control)
Major Investigators: Michael J. Yabsley

December 1, 2016 - February 28, 2020

The goal of this agreement was that the granular level of detail in forecast maps will strongly support the evidence-based use of Lyme disease vaccines.

The objectives of this project were:

- To refine mathematical modeling necessary for forecasts, with emphasis on increasing automation and computational speed of the current United States algorithm.
- To initiate research to quantify the effect of preventative immunization on the regional control of Lyme disease.
- To provide continuing education regarding parasite prevention and use of CAPC forecast maps as a tool for veterinarians to become the local expert in parasite control.
- To assist with quantify changes in the prevalence and geographic distribution of Lyme disease and other tick-borne diseases.

TECHNICAL PUBLICATIONS AUTHORIZED OR CO-AUTHORED BY SCWDS PERSONNEL

Published

Adams, H., M.H. Murray, C. Welch, A. Kidd-Weaver, T. Ellison, S. Curry, J. Hepinstall-Cymerman, and S.M. Hernandez. 2019. Capturing American white ibises in urban South Florida using two novel techniques. *Journal of Field Ornithology* doi: 10.1111/jofo.12311.

ABSTRACT: As many wildlife species, including wading birds, adapt to anthropogenic landscapes and, in some cases, exhibit altered behaviors, studies that involve capturing birds may require new methods better suited for use in urban areas and to accommodate altered animal behavior. We developed two novel techniques, a leg lasso and flip net, for capturing American white ibises (*Eudocimus albus*) in urban environments in southern Florida, and also used a traditional technique (mist-nets) in non-urban wetland habitats. The flip net and leg lasso were developed to capture white ibises habituated to the presence of humans. Ibises were captured in urban and wetland environments from October 2015 to August 2017 in Palm Beach, Broward, and Lee counties, Florida. We captured 6.0 ± 13.5 ibis/h with the flip net, 1.6 ± 0.8 ibis/h with the leg lasso, and 0.5 ± 2.6 ibis/h with mist-nets. We captured larger (higher mass to tarsus length ratio) birds using the flip net and leg lasso than using mist-nets, and captured more males with leg lassos than with other two techniques. The novel techniques we used are efficient, cost effective, easy to use, and also potentially useful for capturing other species of birds. Leg lassos and flip nets are also safe to use in populated areas for both birds and humans.

Allen, S.E., N.A. Vogt, B. Stevens, M.G. Ruder, C.N. Jardine, and N.N. Nemeth. 2020. A retrospective summary of cervid morbidity and mortality in Ontario and Nunavut regions of Canada (1991-2017). *Journal of Wildlife Diseases* 56(4); doi: 10.7589/JWD-D-19-00018.

ABSTRACT: Free-ranging cervids in Canada face diverse threats such as climate change, human population expansion, and the northward spread of vector-borne pathogens. However, we currently have a limited understanding of the impacts of these health challenges in Ontario cervids. Our objective was to identify and characterize causes of morbidity and mortality in free-ranging cervids submitted to the Ontario and Nunavut node of the Canadian Wildlife Health Cooperative (CWHC) over a 27-yr period (1991 to 2017). Submissions included carcasses submitted for full postmortem examination (gross and histopathology; *n*=196) and field-collected tissues (*n*=384). Ancillary tests were performed on a case-by-case basis. Univariable logistic regression was used to test for associations between select causes of morbidity and mortality, and factors such as sex, age, and season. Four cervid species were examined: white-tailed deer (*Odocoileus virginianus*; *n*=211), moose (*Alces*; *n*=140), elk (*Cervus canadensis*; *n*=136), and caribou (*Rangifer tarandus* subspecies; *n*=93). Noninfectious disease was the most common general cause of morbidity and mortality (38.1%; 221/580) and was most commonly attributed to trauma (49.7%; 110/221). Deaths attributed to infectious diseases (34.3%; 199/580) were most often bacterial in etiology (45.7%; 91/199). The most common primary infectious disease diagnosed in caribou was digital limb infection, and moose were most commonly diagnosed with parasitic causes, including meningeal worm (*Parelaphostrongylus tenuis*) and winter tick (*Dermacentor albipictus*). Chronic wasting disease was not diagnosed among cervids tested during the study period, consistent with the previous lack of detection of the disease in Ontario. These results reveal that anthropogenic, infectious, and environmentally associated causes of morbidity and mortality are commonly diagnosed in cervids submitted to the CWHC Ontario and Nunavut regions, and represent potential population threats that should continue to be monitored.

Ayala, A.J., S.M. Hernandez, T.L. Olivier, C.N. Welch, K.M. Dimitrov, I.V. Goraichuk, C.L. Afonso, and P.J. Miller. 2019. Experimental infection and transmission of Newcastle disease vaccine virus in four wild passerines. *Avian Diseases* 63(3): 389-399.

ABSTRACT: Our prior work has shown that live poultry vaccines have been intermittently isolated from wild birds sampled during field surveillance studies for Newcastle disease virus (NDV). Thus, we experimentally investigated the susceptibility of four native agriculturally associated wild bird species to the NDV LaSota vaccine and evaluated the shedding dynamics, potential transmission from chickens, and humoral antibody responses. To test susceptibility, we inoculated wild-caught, immunologically NDV-naïve house finches (*Haemorhous mexicanus*; *n*=16), brown-headed cowbirds (*Molothrus ater*; *n*=9), northern cardinals (*Cardinalis cardinalis*; *n*=6), and American goldfinches (*Spinus tristis*; *n*=12) with 0.1 ml ($10^{6.7}$ mean embryo infectious doses [$EID_{50/ml}$]) of NDV LaSota vaccine via the oculo-nasal route. To test transmission between chickens and wild birds, adult specific-pathogen-free white leghorn chickens were inoculated similarly and cohoused in separate isolators with two to five wild birds of the species listed above. This design resulted in three treatments: wild bird direct inoculation (five groups) and wild bird exposure to one (two groups) or two inoculated chickens (six groups), respectively. Blood and oropharyngeal and cloacal swabs were collected before and after infection with the live vaccine. All wild birds that were directly inoculated with the LaSota vaccine shed virus as demonstrated by virus isolation (VI). Cardinals were the most susceptible species based on shedding viruses from 1 to 11 days postinoculation (dpi) with titers up to $10^{4.9}$ EID_{50}/ml. Although LaSota viruses were shed by all inoculated chickens and were present in the drinking water, most noninoculated wild birds cohoused with these chickens remained uninfected for 14 days as evidenced by VI. However, one American goldfinch tested positive for vaccine transmission by VI at 7 dpi and one house finch tested positive for vaccine transmission by real-time reverse-transcription PCR at 13 dpi. Only one directly inoculated cowbird (out of three) and two cardinals (out of two) developed

NDV-specific hemagglutination inhibition antibody titers of 16, 16, and 128, respectively. No clinical signs were detected in the chickens or the wild birds postinoculation.

Bahnson, C.S., S.M. Hernandez, R.L. Poulson, R.E. Cooper, S.E. Curry, T.J. Ellison, H.D. Adams, C.N. Welch, and D.E. Stallknecht. 2020. Experimental infections and serology indicate that American white ibis (*Eudociumus albus*) are competent reservoirs for type A influenza virus. *Journal of Wildlife Diseases* 56(3): 530-537.

ABSTRACT: The American white ibis (*Eudocimus albus*) is a nomadic wading bird common to wetland habitats in the southeastern U.S. In south Florida, U.S., habitat depletion has driven many ibis to become highly urbanized. Although they forage in neighborhood parks, artificial wetlands, backyards, and golf courses, the majority continue to nest in natural wetlands, often in dense, mixed species colonies. Adults and juveniles commonly disperse thousands of kilometers to other breeding colonies along the Gulf and southeast Atlantic coasts, presenting the potential for close contact with humans, domestic animals, and other wild bird species. Historically, wading birds were not considered to be significant hosts for influenza A virus (IAV), yet as ibis regularly move among various human, domestic animal, and wildlife interfaces, their potential to be exposed to or infected with IAV deserves attention. We experimentally challenged wild-caught, captive-reared white ibis (*n*=20) with IAV, tested wild white ibis for IAV, and serologically tested wild white ibis for antibodies to IAV. White ibis were highly susceptible to experimental challenge with H6N1 and H11N9 IAVs, with cloacal shedding lasting an average of 6 d. All 13 infected birds seroconverted by 14 d postinfection as determined by microneutralization. In contrast, no birds challenged with H3N8 were infected. We tested 118 swabs and 578 serum samples from white ibis captured in southeastern Florida for IAV infection and antibodies to IAV, respectively. Although no IAVs were isolated, 70.4% serum samples were antibody positive by blocking enzyme-linked immunosorbent assay (bELISA). Neutralizing antibodies to H1–H12 were detected in 96.0% of a subset of bELISA positive birds (*n*=196) and 81.0% tested antibody positive to two or more hemagglutinin subtypes, indicating that exposure to multiple IAVs is common. These results provide evidence that white ibis are susceptible and naturally infected with IAV and may represent a component of the IAV natural reservoir system.

Bahnson, C.S., R.L. Poulson, L.P. Hollander, J.C. Bradley, and D.E. Stallknecht. 2020. Susceptibility of laughing gulls (*Leucophaeus atricilla*) and mallards (*Anas platyrhynchos*) to ruddy turnstone (*Arenaria interpres morinella*) origin type A influenza viruses. *Journal of Wildlife Diseases* 56(1): 167-174.

ABSTRACT: Delaware Bay, U.S., is the only documented location where influenza A virus (IAV) is consistently detected in a shorebird species, the ruddy turnstone (RUTU; *Arenaria interpres morinella*). Although IAV in shorebirds has been well studied at this site for decades, the importance of other species in the avian community as potential sources for the IAVs that infect RUTUs each spring remains unclear. We determined the susceptibility of mallards (*Anas platyrhynchos*) and laughing gulls (*Leucophaeus atricilla*), to IAVs isolated from RUTUs in order to gain insight into the potential host range of these viruses. Captive-reared gulls were challenged with RUTU-origin H6N1, H10N7, H11N9, H12N4, and H13N6 IAV, as well as mallard-origin H6N1 and H11N9. We challenged captive-reared mallards with the same viruses, except for H13N6. At a biologically plausible challenge dose (10^4 50% embryo infective doses/0.1 mL), one of five gulls challenged with both H6N1 IAVs shed virus. The remaining gulls were resistant to infection with all viruses. In contrast, all mallards were infected and shed virus. The H12N4 mallard challenge group was an exception with no birds infected. These results indicated that mallards are permissive to infection with viruses

originating from a shorebird host and that interspecies transmission could occur. In contrast, host adaptation of IAVs to RUTUs may compromise their ability to be transmitted back to gulls.

Bloodgood, J.C.G., S.M. Hernandez, A. Isaiah, J.S. Suchodolski, L.A. Hoopes, P.M. Thompson, T.B. Waltzek, and T.M. Norton. 2020. The effect of diet on the gastrointestinal microbiome of juvenile rehabilitating green turtles (*Chelonia mydas*). *PloS One* 15(1): e0227060.

ABSTRACT: Threatened and endangered green turtles (*Chelonia mydas*) are unique because as juveniles they recruit from pelagic to near-shore waters and shift from an omnivorous to primarily herbivorous diet (i.e. seagrass and algae). Nevertheless, when injured and ill animals are admitted to rehabilitation, animal protein (e.g. seafood) is often offered to combat poor appetite and emaciation. We examined how the fecal microbiome of juvenile green turtles changed in response to a dietary shift during rehabilitation. We collected fecal samples from January 2014-January 2016 from turtles (N=17) in rehabilitation at the Georgia Sea Turtle Center and used next generation sequencing to analyze bacterial community composition. Samples were collected at admission, mid-rehabilitation, and recovery, which entailed a shift from a mixed seafood–vegetable diet at admission to a primarily herbivorous diet at recovery. The dominant phyla changed over time, from primarily Firmicutes (55.0%) with less Bacteroidetes (11.4%) at admission, to primarily Bacteroidetes (38.4%) and less Firmicutes (31.8%) at recovery. While the microbiome likely shifts with the changing health status of individuals, this consistent inversion of Bacteroidetes and Firmicutes among individuals likely reflects the increased need for protein digestion, for which Bacteroidetes are important. Firmicutes are significant in metabolizing plant polysaccharides; thus, fewer Firmicutes may result in underutilization of wild diet items in released individuals. This study demonstrates the importance of transitioning rehabilitating green turtles to an herbivorous diet as soon as possible to afford them the best probability of survival.

Calvente, E., N. Chinnici, J.D. Brown, J.E. Banfield, J.W. Brooks, and M.J. Yabsley. 2020. Winter tick (*Dermacentor albipictus*)-associated dermatitis in a wild elk (*Cervus canadensis*) in Pennsylvania, USA. *Journal of Wildlife Diseases* 56(1): 247-250.

ABSTRACT: In 2017, a male elk (*Cervus canadensis*) was found dead in Pennsylvania, U.S. The elk was in poor nutritional condition and had alopecia and ulcerative dermatitis throughout the neck and dorsum region associated with severe *Dermacentor albipictus* infestations. Histologically, there was severe chronic-active dermatitis with hyperkeratosis and crust formation.

Chambouvet, A., V. Smilansky, M. Jirku, M. Isidora-Ayza, S. Itoiz, E. Derrelle, A. Monier, D.J. Gower, M. Wilkinson, M.J. Yabsley, J. Lukes, and T.A. Richards. 2020. Diverse alveolate infections of tadpoles, a new threat to frogs? *Plos Pathogens* 16(2): e1008107.

ABSTRACT: Amphibians are one of the most threatened major groups of animals, with decline in amphibian populations often cited as support for the claim that we are witnessing a mass extinction event. The following causes of amphibian decline have been suggested: 1) invasive species causing ecosystem change, 2) overexploitation of natural environments, 3) changes in land use, 4) global environmental change, such as global warming, 5) increased use of pesticides and other polluting chemicals, and 6) the emergence and/or spread of infectious diseases. We need to consider all of these factors if we are to understand amphibian decline and plan conservation strategies accordingly.

Christensen, S.A., M.G. Ruder, D.M. Williams, W.F. Porter, and D.E. Stallknecht. 2020. The role of drought as a determinant of hemorrhagic disease in the eastern United States. *Global Change Biology* 26(7): 3799-3808.

ABSTRACT: Bluetongue virus and epizootic hemorrhagic disease (HD) virus are globally distributed, vector-borne viruses that infect and cause disease in domestic and wild ruminant species. The forces driving increases in resulting HD may be linked to weather conditions and increasing severity has been noted in northerly latitudes. We evaluated the role of drought severity in both space and time on changes in HD reports across the eastern United States for a recent 15 year period. The objectives of this study were to: (a) develop a spatiotemporal model to evaluate if drought severity explains changing patterns of HD presence; and (b) determine whether this potential risk factor varies in importance over the present range of HD in the eastern United States. Historic data (2000–2014) from an annual HD presence–absence survey conducted by the Southeastern Cooperative Wildlife Disease Study and from the United States Drought Monitor were used for this analysis. For every county in 23 states and for each of 15 years, data were based on reported drought status for August, wetland cover, the physiographic region, and the status of HD in the previous year. We used a generalized linear mixed model to explain HD presence and evaluated spatiotemporal predictors across the region. We found that drought severity was a significant predictor of HD presence and the significance of this relationship was dependent on latitude. In more northerly latitudes, where immunological naivety is most likely, we demonstrated the increasing strength of drought severity as a determinant of reported HD and established the importance of variation in drought severity as a risk factor over the present range of HD in the eastern United States. Our research provides spatially explicit evidence for the link between climate forces and emerging disease patterns across latitude for a globally distributed disease.

Cleveland, C.A., K.B. Garrett, E.K. Box, Z. Eure, A.A. Majewska, J.A. Wilson, and M.J. Yabsley. 2020. Cooking copepods; the survival of cyclopoid copepods (Crustacea; Copepoda) in simulated provisioned water containers and implications for the Guinea Worm Eradication Program in Chad, Africa. *International Journal of Infectious Diseases* 95: 216-220; doi: 10.1016/j.ijid.2020.03.016.

ABSTRACT: The global Guinea Worm Eradication Program has reduced numbers of human infections of Guinea worm disease (dracunculiasis) to 49 cases in four countries. However, infections of domestic animals (dogs and cats) have recently been recognized and are increasing. Typically, Guinea worm (*Dracunculus medinensis*) transmission occurs via the ingestion of copepods from water. Despite several interventions, including tethering of dogs while worms emerge, the number of infected dogs continue to increase. One hypothesis is that dogs could be infected through the ingestion of copepods in provisioned water. The purpose of this study was to determine whether copepods can survive in water containers under typical Chadian temperatures. Four container types (plastic, glass, gourd, and metal) were seeded with copepods and exposed to simulated Chadian temperatures. All copepods in the metal containers died within 4 h. Conversely, after 8 h live copepods were still present in plastic, glass, and gourd containers. If provisioned water is provided to potential hosts of *D. medinensis*, metal containers create the most inhospitable environment for copepods. Plastic containers have little effect on copepod mortality. The use of metal containers for water provisions could be a useful tool assisting with the interruption of *D. medinensis* transmission among dogs.

Cozad, R.A., S.M. Hernandez, T.M. Norton, T.D. Tuberville, N.I. Stacy, N.L. Stedman, and M.J. Aresco. 2020. Epidemiological investigation of a mortality event in a translocated gopher tortoise (*Gopherus polyphemus*) population in northwest Florida. *Frontiers in Veterinary Science* https://doi.org/10.3389/fvets.2020.00120.

ABSTRACT: Nokuse Plantation, a 22,055 ha private conservation preserve in northwest Florida, is a recipient site for gopher tortoises translocated from development sites in Florida. Since 2006, Nokuse has received over 5,000 tortoises from multiple development sites. During 2013-2015, 52 tortoises were found sick (*n*=14) or dead (*n*=38) in multiple soft-release enclosures in which tortoises consistently exhibited clinical signs, with additional sick (*n*=5) and dead (*n*=5) tortoises presenting similarly during 2016–2017. When found alive, tortoises behaved abnormally (e.g., frequently out of burrows during cold weather, pacing along enclosure fencing), appeared emaciated, were lethargic, and had developed redness under plastron scutes. Similar numbers of male (*n*=28) and female (*n*=32) tortoises were recovered along with two of unidentified sex, including mainly adults (*n*=59) and three subadults. Physical examination, blood analysis, and other diagnostics were indicative of starvation and dehydration. Most sick tortoises provided with supportive care recovered. Necropsy findings generally confirmed starvation, with no evidence of infectious pathogens or contaminants. There were no apparent differences in quality of habitat, plant community, or soil or water among affected and unaffected enclosures. Botanical surveys indicated adequate forage quality and quantity, with no poisonous exotic or native plants detected. No land management practices changed prior to this event. Analysis of epidemiological data and demographic factors from before and during this mortality event identified initial density of tortoises in the enclosures as exerting the strongest influence on detection of tortoise morbidity and mortality. We believe that the stress associated with mixing tortoises from different populations and at higher densities during translocation impacted an individual tortoise's ability to obtain or absorb adequate nutrients from foraging, ultimately leading to a wasting condition consistent with starvation. Based on our findings, we recommend a maximum of three gopher tortoises per ha in soft-release enclosures for translocation, but further research is warranted to investigate the complexity of stress and social pressures associated with translocation.

Cozad, R.A., T.M. Norton, M.J. Aresco, M.C. Allender, and S.M. Hernandez. 2020. Pathogen surveillance and detection of ranavirus (*Frog virus 3*) in translocated gopher tortoises (*Gopherus polyphemus*). *Journal of Wildlife Diseases* 56(3): 679-683.

ABSTRACT: Emerging pathogens may pose additional threats to already vulnerable populations of chelonians, such as gopher tortoises (*Gopherus polyphemus*). In response to a mortality event on a translocation site in northwest Florida during 2013-2015, 13 gopher tortoises were necropsied and their tissues were screened for 12 pathogens, including *Mycoplasma agassizii*, *Mycoplasma testudineum*, and *Frog virus 3*-like ranavirus (FV3). The DNA of FV3 was detected via quantitative PCR in the gastrointestinal tract of three tortoises. Subsequently, pathogen surveillance was performed on whole blood and oral-cloacal swab samples of live translocated tortoises from two different enclosures within the site (*n*=68), rehabilitated tortoises from the site (*n*=18), and tortoises prior to release on site (*n*=35) during 2015-2017. *Mycoplasma* spp. were present in all groups and years of live tortoises tested. The DNA of FV3 was detected in 15 individuals both with and without clinical signs of disease in 2016. We recaptured 20 tortoises and captured an additional 20 tortoises in 2017 for surveillance, yet FV3 DNA was no longer detected, even in those that had previously tested positive (*n*=7). The results of this study contribute to the epidemiology of ranavirus in chelonians and suggests that gopher tortoises could be reservoirs for FV3. We recommend that the status of *Ranavirus* infection should be included for health screens for gopher tortoises in translocation programs.

Cummings, C.R., S.M. Hernandez, M. Murray, T. Ellison, H.C. Adams, R.E. Cooper, S. Curry, and K.J. Navara. 2020 Effects of an anthropogenic diet on indicators of physiological challenge and immunity of white ibis nestlings raised in captivity. *Ecology and Evolution* doi.org/10.1002/ece3.6548.

ABSTRACT: When wildlife forage and/or live in urban habitats, they often experience a shift in resource availability and dietary quality. Some species even use human handouts, such as bread, as well as human refuse, as a large part of their new diets; yet the influences of this nutritional shift on health and survival remain unclear. American white ibises are increasingly being seen in urban areas in Florida; they collect handouts, such as bread and other food items, from humans in parks, and are also found foraging on anthropogenic sources in trash heaps. We hypothesized that the consumption of these new anthropogenic food sources may trigger increases in indicators of physiological challenge and dampen immune responses. We tested this experimentally by raising 20 white ibis nestlings in captivity, and exposing 10 to a simulated anthropogenic diet (including the addition of white bread and a reduction in seafood content) while maintaining 10 on a diet similar to what ibises consume in more natural environments. We then tested two indicators of physiological challenge (corticosterone and heat shock protein 70), assessed innate immunity in these birds via bactericidal assays and an in vitro carbon clearance assay, and adaptive immunity using a phytohemagglutinin skin test. The anthropogenic diet depressed the development of the ability to kill *Salmonella paratyphi* in culture. Our results suggest that consuming an anthropogenic diet may be detrimental in terms of the ability to battle a pathogenic bacterial species, but there was little effect on indicators of physiological challenge and other immunological measures.

Diskin, E.R., K. Friedman, S. Krauss, J.M. Nolting, R.L. Poulson, R.D. Slemons, D.E. Stallknecht, R.G. Webster, and A.S. Bowman. 2020. Subtype diversity of influenza A virus in North American waterfowl: a multi-decade study. *Journal of Virology* 94(11): e02022-19.

ABSTRACT: The discovery in 1976 of waterfowl as the primary reservoir of influenza A viruses (IAVs) has since spurred decades of waterfowl surveillance efforts by researchers dedicated to understanding the ecology of IAV and its subsequent threat to human and animal health. Here, we employed a multidecade, continental-scale approach of surveillance data to understand trends of seasonal IAV subtype diversity. Between 1976 and 2015, IAVs were detected in 8,427 (10.8%) of 77,969 samples from migratory waterfowl throughout the Central and Mississippi Migratory Flyways in the United States and Canada. A total of 96 hemagglutinin (HA)/neuraminidase (NA) subtype combinations were isolated, which included most HA (H1 to H14) and all 9 NA subtypes. We observed an annual trend of high influenza prevalence, involving a few dominant subtypes, on northern breeding grounds during summer with progressively lowered influenza prevalence, comprised of a highly diverse profile of subtypes, as waterfowl migrate toward southern wintering grounds. Isolates recovered during winter had the highest proportion of mixed and rare HA/NA combinations, indicating increased opportunity for reassortment of IAVs. In addition, 70% of H5 and 49% of H7 IAV isolates were recovered from samples collected during fall and spring, respectively; these are subtypes that can have significant implications for public health and agriculture sectors. Annual cyclical dominance of subtypes on northern breeding grounds is revealed through the longitudinal nature of this study. Our novel findings exhibit the unrealized potential for discovery using existing IAV surveillance data.

Ford, A.K., K.D. Niedringhaus, A.N. Anderson, J.M. LaCour, and N.M. Nemeth. 2020. Disseminated *Mycobacterium kansasii* infection in a white-tailed deer and implications for public and livestock health. *Journal of Veterinary Diagnostic Investigation* 32(1): 147-151.

ABSTRACT: We document a case of *Mycobacterium kansasii*, a rare, zoonotic bacterium, in a white-tailed deer (WTD; *Odocoileus virginianus*) in East Baton Rouge Parish, Louisiana. Grossly, the deer had fibrinous pleuropneumonia with yellow, mineralized nodules scattered throughout the lungs and extending to the pleura. The kidneys were enlarged and had numerous pale foci in the cortex. Microscopically, the pulmonary architecture was replaced by variably sized, multifocal-to-coalescing granulomas with peripheral histiocytes and fewer multinucleate giant cells, and necrotic centers with mineralization and hemorrhage. The latter rarely contained one to a few acid-fast, slender, 7-µm long bacteria, for which beaded morphology was sometimes evident. Similar acid-fast bacteria were also within histiocytes in the kidney. PCR assay of fresh lung sample and subsequent sequencing revealed a non-tuberculosis mycobacterium, *M. kansasii*. These lesions were similar to those that result from infection with *M. bovis* in WTD. Both *M. bovis* and *M. kansasii* are zoonotic. WTD are a reservoir of *M. bovis*, which is a major concern in regions in which WTD and cattle can come into close contact.

Ganoe, L.S., J.D. Brown, M.J. Yabsley, M.J. Lavallo, and W.D. Walter. 2020. A review of pathogens, diseases, and contaminants of muskrats (*Ondatra zibethicus*) in North America. *Frontiers in Veterinary Science* 7: 233.

ABSTRACT: Over the last 50 years, significant muskrat (*Ondatra zibethicus*) harvest declines have been observed throughout North America. Several theories for the decline have been proposed, including increased parasite infections and disease within muskrat populations. No existing wholistic review of muskrat exposure to pathogens, contaminants, and diseases exists. To address this knowledge gap, we conducted a thorough review of existing literature on muskrat pathogens, contaminants, and diseases across their natural range. This review is comprised of 131 articles from 1915 to 2019 and from 27 U.S. states and 9 Canadian provinces. A wide diversity of contaminants, toxins, and pathogens were reported in muskrats, with the most common diseases being cysticercosis, tularemia, Tyzzer's disease, and biotoxin poisoning from cyanobacteria. This review provides a summary of muskrat pathogens, contaminants, and diseases over a century that has observed significant population declines throughout the species' range in North America. Such data provide a baseline for understanding the potential role of disease in these declines. In addition, these data highlight critical knowledge gaps that warrant future research efforts.

Garrett, K.B., E.K. Box, C.A. Cleveland, A.A. Majewska, and M.J. Yabsley. 2020. Dogs and the classic route of Guinea Worm transmission: an evaluation of copepod ingestion. *Scientific Reports* 10(1): 1430.

ABSTRACT: *Dracunculus medinensis*, the causative agent of Guinea worm disease in humans, is being reported with increasing frequency in dogs. However, the route(s) of transmission to dogs is still poorly understood. Classical transmission to humans occurs via drinking water that contains cyclopoid copepods infected with third stage larvae of *D. medinensis*, but due to the method of dog drinking (lapping) compared to humans (suction and/or retrieval of water into containers), it seems unlikely that dogs would ingest copepods readily through drinking. We exposed lab raised beagles to varying densities of uninfected copepods in 2 liters of water to evaluate the number of copepods ingested during a drinking event. We confirmed dogs can ingest copepod intermediate hosts while drinking; however, low numbers were ingested at the densities that are typically observed in Chad suggesting this transmission route may be unlikely. Overall, the relative importance of the classic transmission route and alternate transmission routes, such as paratenic and transport hosts, needs investigation in order to further clarify the epidemiology of guinea worm infections in dogs.

Gettings, J.R., S.C. Self, C.S. McMahan, D.A. Brown, S.K. Nordone, and M.J. Yabsley. 2020. Local and regional temporal trends (2013-2019) of canine *Ehrlichia* spp. seroprevalence in the USA. *Parasite Vectors* 13: 153; doi: 10.1186/s13071-020-04022-4.

ABSTRACT: In the USA, there are several *Ehrlichia* spp. of concern including *Ehrlichia canis*, *Ehrlichia ewingii*, *Ehrlichia chaffeensis*, *Ehrlichia muris eauclarensis*, and “Panola Mountain *Ehrlichia*”. Of these, *E. canis* is considered the most clinically relevant for domestic dogs, with infection capable of causing acute, subclinical, and chronic stages of disease. Changes in climate, land use, habitats, and wildlife reservoir populations, and increasing contact between both human and dog populations with natural areas have resulted in the increased risk of vector-borne disease throughout the world. A Bayesian spatio-temporal binomial regression model was applied to serological test results collected from veterinarians throughout the contiguous USA between January 2013 and November 2019. The model was used to quantify both regional and local temporal trends of canine *Ehrlichia* spp. seroprevalence and identify areas that experienced significant increases in seroprevalence. Regionally, increasing seroprevalence occurred within several states throughout the central and southeastern states, including Missouri, Arkansas, Mississippi, Alabama, Virginia, North Carolina, Georgia and Texas. The underlying local trends revealed increasing seroprevalence at a finer scale. Clusters of locally increasing seroprevalence were seen from the western Appalachian region into the southern Midwest, along the Atlantic coast in New England, parts of Florida, Illinois, Wisconsin and Minnesota, and in a couple areas of the Mountain region. Clusters of locally decreasing seroprevalence were seen throughout the USA including New York and the mid-Atlantic states, Texas, the Midwest, and California. Canine *Ehrlichia* spp. seroprevalence is increasing in both endemic and non-endemic areas of the USA. The findings from this study indicate that dogs across a wide area of the USA are at risk of exposure and these results should provide veterinarians and pet owners with the information they need to make informed decisions about prevention of tick exposure.

Gibson, D.J., N.M. Nemeth, H. Beaufrère, C. Varga, D. Ojkic, A. Marom, and L. Susta. 2019. Development and use of a triplex real-time PCR assay for detection of three DNA viruses in psittacine birds. *Journal of Veterinary Diagnostic Investigation* 31(5): 719-725.

ABSTRACT: Aves polyomavirus 1, psittacine beak and feather disease virus, and psittacid herpesvirus 1 are important pathogens of psittacine birds with the potential to cause substantial morbidity and mortality. Using publically available nucleotide sequences, we developed and validated a triplex real-time PCR (rtPCR) assay to rapidly detect these 3 viruses. The assay had high analytical sensitivity, detecting <6 copies of viral DNA per reaction, and 100% analytical specificity, showing no cross-reactivity with 59 other animal pathogens. Archived formalin-fixed, paraffin-embedded tissues from psittacine birds diagnosed at postmortem as infected with each of the viruses as well as virus-negative birds were used to validate the utility of the assay. Birds were selected for the positive cohort if they showed histologic evidence of infection (i.e., characteristic inclusion bodies in tissues); birds in the negative cohort had final diagnoses unrelated to the pathogens of interest. The triplex rtPCR assay confirmed 98% of histopathology-positive cases, and also identified subclinical infections that were not observed by histologic examination, including coinfections. Birds that tested positive only by rtPCR had significantly higher cycle threshold values compared to those with histologic evidence of infection. Positive, negative, and overall percentage agreements as well as the kappa statistic between the results of the assay and histopathology were high, demonstrating the usefulness of the assay as a tool to confirm disease diagnoses, and to improve detection of subclinical infections.

Greenawalt, D., P. Dunn, J.D. Brown, M.J. Yabsley, L. Williams, M.J. Casalena, R. Boyd, E. Debelak, H. Wildicka, E. Phillips, and E. Wallner-Pendleton. 2020. Surveillance for *Heterakis* spp. in game birds and cage-free, floor-raised poultry in Pennsylvania, USA. *Avian Diseases* 64(2): 210-215.

ABSTRACT: Histomoniasis is a significant disease of gallinaceous birds caused by *Histomonas meleagridis*. Transmission of this parasite is dependent on use of the cecal nematode *Heterakis gallinarum*. To define the host range of this nematode, cecal contents from 399 game birds and poultry, representing eight species, were examined for *Heterakis* spp. The majority of these species (five of eight) were infected with *Heterakis* nematodes. *Heterakis gallinarum* was detected in free-ranging wild turkeys (*Meleagridis gallopovo*), captive-raised ring-necked pheasants (*Phasianus colchicus*), chukars (*Alectoris chukar*), and domestic chickens (*Gallus domesticus*), whereas *H. isolonche* was found in ruffed grouse (*Bonasa umbellus*). No *Heterakis* species were identified in the domestic turkey (*Meleagridis gallopovo*), American woodcock (*Scolopax minor*), and dabbling duck (*Anas* spp.) samples. Genetic characterization indicated that nematodes identified as *H. gallinarum* were present in two distinct clades. One clade of *H. gallinarum* sequenced from this study grouped with chicken-derived sequences from other countries. The other group of sequences consisted of a sister clade to a group of parasites morphologically identified as *H. isolonche*. Currently it is unknown if this group represents a genetic variant of *H. gallinarum*, a variant of *H. isolonche*, or a novel species. These results indicate *Heterakis* infection varies among poultry and game bird species but is common among select gallinaceous species in Pennsylvania.

Groves, B.A., M.J. Yabsley, L. Swanepoel, and M.M. Garner. 2020. Lungworm (*Crenosoma goblel*) infection in unweaned free-ranging raccoons (*Procyon lotor*) in Washington State, USA. *Journal of Wildlife Diseases* 56(2): 419-423.

ABSTRACT: Larval parasites morphologically identified as *Crenosoma goblei* were found in orphaned raccoons (*Procyon lotor*) admitted to a wildlife rehabilitation center in Washington State, U.S. Partial 18S rRNA and cytochrome oxidase I gene sequences were most similar to other *Crenosoma* spp. Although commonly reported in free-ranging raccoons throughout much of the central and eastern US, this finding extends the range of *C. goblei* into the Pacific Northwest of North America. Fecal samples taken within 48 h of admission were positive for larvae in 70% (21/30) of individuals and in all five group samples taken from an additional 14 raccoons. Typically, *Crenosoma* spp. use an intermediate host, but the unweaned status of all the raccoons in this report suggests a potential direct, possibly vertical, route of transmission. Serial fecal analysis of pooled samples collected over several subsequent months demonstrated spontaneous resolution of larval shedding. Adult worms and gross evidence of pneumonia were detected in the lungs of several juvenile raccoons at necropsy, and verminous pneumonia was confirmed histologically in the single case submitted. Clinical signs directly attributable to verminous pneumonia were not seen in most actively shedding raccoons, suggesting that subclinical disease among juveniles may be common.

Huckins, G.L., D. Eschar, D. Schwarz, M. Morton, B.H. Herrin, A. Cerezo, M.J. Yabsley, and S.M. Schneider. 2019. *Trypanosoma cruzi* infection in zoo-housed red panda in Kansas. *Journal of Veterinary Diagnostic Investigation* 31(5): 752-755.

ABSTRACT: A 9-y-old, zoo-housed, male red panda (*Ailurus fulgens*) became progressively lethargic and inappetent over a 1-wk period. Physical examination was unremarkable. A complete blood count showed mild normocytic, normochromic, non-regenerative anemia with the presence of trypomastigote organisms, consistent with a *Trypanosoma* sp. The organism was confirmed later as *Trypanosoma cruzi* lineage TcI via PCR and genome sequencing. The

panda was initially treated supportively; however, its clinical status within 24 h from presentation deteriorated, and euthanasia was elected. Autopsy showed severe systemic *T. cruzi* infection with the presence of amastigotes in the heart, brain, peripheral nerves, skeletal muscles, tongue, liver, and testes. We used genome sequencing and serology in identifying the agent.

Jessup, D.A., and S.M. Hernandez. 2020. Decline in North American wild bird populations. Letters to the Editor. *Journal of the American Veterinary Medical Association* 256(7): 761-765.

Jessup, D.A., and S.M. Hernandez. 2020. Further discussion on the decline in North American wild bird populations. Letters to the Editor. *Journal of the American Veterinary Medical Association* 257(2): 144-145.

Jia, B., A. Colling, D.E. Stallknecht, D. Biehert, J. Bingham, B. Crossley, D. Eagles, and I.A. Gardner. 2020. Validation of laboratory tests for infectious diseases in wild mammals: review and recommendations. *Journal of Veterinary Diagnostic Investigation* p. 1040638720920346, doi: 10.1177/1040638720920346.

ABSTRACT: Evaluation of the diagnostic sensitivity (DSe) and specificity (DSp) of tests for infectious diseases in wild animals is challenging, and some of the limitations may affect compliance with the OIE-recommended test validation pathway. We conducted a methodologic review of test validation studies for OIE-listed diseases in wild mammals published between 2008 and 2017 and focused on study design, statistical analysis, and reporting of results. Most published papers addressed *Mycobacterium bovis* infection in one or more wildlife species. Our review revealed limitations or missing information about sampled animals, identification criteria for positive and negative samples (case definition), representativeness of source and target populations, and species in the study, as well as information identifying animals sampled for calculations of DSe and DSp as naturally infected captive, free-ranging, or experimentally challenged animals. The deficiencies may have reflected omissions in reporting rather than design flaws, although lack of random sampling might have induced bias in estimates of DSe and DSp. We used case studies of validation of tests for hemorrhagic diseases in deer and white-nose syndrome in hibernating bats to demonstrate approaches for validation when new pathogen serotypes or genotypes are detected and diagnostic algorithms are changed, and how purposes of tests evolve together with the evolution of the pathogen after identification. We describe potential benefits of experimental challenge studies for obtaining DSe and DSp estimates, methods to maintain sample integrity, and Bayesian latent class models for statistical analysis. We make recommendations for improvements in future studies of detection test accuracy in wild mammals.

Kidd-Weaver, A., J. Hepinstall-Cymerman, C.N. Welch, M.H. Murray, H.C. Adams, T.J. Ellison, M.J. Yabsley, and S.M. Hernandez. 2020. The movements of a recently urbanized wading bird reveal changes in season timing and length related to resource use. *PLoS One* 15(3): e0230158.

ABSTRACT: The American white ibis (*Eudocimus albus*) is a nomadic wading bird that is increasing the amount of time spent foraging in urban areas, relying on artificial wetlands and other anthropogenic resources year-round. In this study, we explore whether and how American white ibis association with urban environments is predictive of variation in the timing and length of behavioral seasons. Other urbanized species exhibit altered annual cycles such as loss of migratory behavior and year-round breeding related to consistent resource abundance, often related to intentional and unintentional provisioning. To determine if these same patterns of behavior were also present in White Ibis, we used behavioral change point analysis to segment the tracks of 41 ibis equipped with GPS backpacks to identify the initiation and duration of four behavioral seasons (non-breeding, pre-breeding, breeding, post-breeding) the degree of urban association. We found that intraspecific variation in urban habitat use had strong carryover effects on the timing and duration of behavioral seasons. This study revealed ibis with higher use of urban habitats in non-breeding seasons had longer non-breeding seasons and shorter breeding seasons that began earlier in the year compared to ibis that primarily use wetland habitats. The timing and duration of seasons also varied with ibis age, such that ibis spent more time engaged in breeding-related seasons as they aged. Juvenile and subadult ibis, though considered to be reproductively immature, also exhibit behavioral shifts in relation to breeding seasons. The behavioral patterns found in this study provide evidence that ibis are adapting their annual cycles and seasonal behaviors to exploit urban resources. Future research is needed to identify the effect of interactions between ibis urban association and age on behavioral season expression.

Larouche, C., H. Beaufrere, C. Mosley, N. Nemeth, and C. Dutton. 2019. Evaluation of the effects of midazolam and flumazenil in the ball python (*Python regius*). *Journal of Zoo and Wildlife Medicine* 50(3): 579-588.

ABSTRACT: The study objective was to evaluate the sedative, muscle relaxant, and cardiorespiratory effects of midazolam and flumazenil in the ball python (*Python regius*). Ten healthy adult female ball pythons were used in a randomized and blinded crossover trial evaluating the effects of two dosages (1 and 2 mg/kg intramuscular [i.m.] in the cranial third of the body). In a subsequent open trial, nine ball pythons received 1 mg/kg i.m. of midazolam followed by 0.08 mg/kg i.m. of flumazenil 60 min later. Heart rate, respiratory rate, temperature, and the level of sedation and muscle relaxation (using a semiobjective scoring system) were evaluated. There were no significant differences between midazolam dosages for any of the parameters evaluated. Sedation scores were significantly increased compared with baseline from 15 min (1 mg/kg) and 10 min (2 mg/kg) postinjection up until 56 hr (1 mg/kg) and 72 hr (2 mg/kg) postinjection. Peak effect was reached 60 min postinjection, with 60% of snakes (6/10) being unable to right themselves. One snake developed paradoxical excitation with the 2 mg/kg dosage. Heart rates were significantly lower than baseline from 30 min to 128 hr postinjection with both midazolam dosages. Respiratory rates were significantly lower than baseline at four time points, with the highest dosage only: 15, 45, 60 min, and 8 hr postinjection. Flumazenil resulted in reversal of sedation and muscle relaxation in all snakes within 10 min of administration. However, resedation was evident in all snakes 3 hr after reversal. Midazolam administered at 1 and 2 mg/kg i.m. provides a moderate to profound, although prolonged, sedation and muscle relaxation in ball pythons. Flumazenil reverses the effects of midazolam in ball pythons, but its duration of action at the evaluated dosage is much shorter than midazolam, leading to resedation.

Larouche, C, J. Beeler-Marfisi, L. Attard, N. Nemeth, and H. Beaufrere. 2019. Hemolymph cytology, hemocyte count, and electrolyte reference values in 93 Cameroon red tarantulas (*Hysterocrates gigas*). *Veterinary Clinical Pathology* 48(3): 461-468.

ABSTRACT: Theraphosidae is a large family of tarantula spiders commonly kept as pets or display animals by zoological institutions. Interest and demand for arachnid medicine have grown over the last decade. While hemolymph analysis could be a fundamental tool for arachnid health assessment, RIs are generally lacking for arachnid species. The objectives of this study were to describe the appearance of hemocytes and establish RIs for total and differential hemocyte counts as well as hemolymph glucose and electrolyte concentrations in the Cameroon red tarantula (CRT) (*Hysterocrates gigas*). Ninety-three CRTs were

anesthetized using isoflurane or sevoflurane, and hemolymph was collected from the heart. Hemocyte counts were performed using a hemocytometer and trypan blue stain, and differential cell counts were estimated using light microscopy and a Wright stain. Hemocytes were also characterized with transmission electron microscopy (TEM). Hemolymph glucose and electrolyte concentrations were obtained using a blood gas analyzer. The American Society for Veterinary Clinical Pathology (ASVCP) guidelines were used to calculate RIs. The most abundant hemocytes were plasmatocytes, followed by granulocytes, cyanocytes, and prohemocytes. Hemocytes were characterized by TEM except for cyanocytes. All RIs had a broad range, likely due to a combination of population heterogeneity, high biologic variability of the species, and the propensity of samples to clot. The current study is the first to establish RIs for hemolymph cytologic parameters with a sample size of over 20 individuals in any theraphosid species. RIs for CRT hemolymph biochemical analytes are also reported.

MacDonald, A.M., J.R. Barta, M. McKay, S. Lair, R. Le Net, F. Baldwin, N. Pople, and N.M. Nemeth. 2019. Lymphoproliferative disease virus in wild turkeys (*Meleagris gallopavo*) from Manitoba and Quebec, Canada. *Avian Diseases* 63(3): 506-510.

ABSTRACT: This study describes the first recognized clinical case of lymphoproliferative disease virus (LPDV) in Canada and extends the range of LPDV in Canada through its detection in Manitoba and Quebec. We assessed the prevalence of LPDV in eastern wild turkeys (*Meleagris gallopavo silvestris*) with the use of whole, clotted blood from live birds in Manitoba (*n*=65) and tissue samples collected postmortem in Quebec (*n*=4). We tested for LPDV proviral DNA through PCR amplification and sequencing of a portion of the *gag* (p31) gene. Samples were also tested for reticuloendotheliosis virus (REV) by PCR. Twenty-four birds (34.8%) were positive for LPDV, including all diagnostic cases. One bird (1.4%) from Quebec had gross and microscopic lesions consistent with LPDV. Two turkeys (2.9%) were REV positive, one (1.4%) of which was co-infected with LPDV. Phylogenetic analysis of LPDV strains from Quebec and Manitoba grouped with previously sequenced samples from Ontario and publicly available sequences from a North American lineage. This study contributes valuable information toward ongoing surveillance and monitoring of LPDV in North America.

MacDonald A.M., D.J. Gibson, J.R. Barta, R. Poulson, J.D. Brown, A.B. Allison, and N.M. Nemeth. 2019. Bayesian phylogenetic analysis of avipoxviruses from North American wild birds demonstrates new insights into host specificity and interspecies transmission. *Avian Diseases* 63(3): 427-432.

ABSTRACT: Avian pox is commonly diagnosed in a variety of North American wild and domestic birds, yet little is known about the evolutionary relationships among the causative poxviruses. This study aimed to determine the phylogenetic relationships among isolates identified in different avian host species to better characterize the host range of specific viral strains and compare the genetic variability within and between viral clades. Skin lesions grossly and microscopically consistent with poxvirus infection from 82 birds collected in Canada, the United States, and the U.S. Virgin Islands were included in this study. A total of 12 avian species were represented; the most common species sampled were wild turkeys (*Meleagris gallopavo*), mourning doves (*Zenaida macroura*), and American crows (*Corvus brachyrhynchos*). Poxvirus samples from these birds were genotyped using PCR that targeted the 4b core protein gene followed by amplicon sequencing. Bayesian phylogenetic analyses of these viruses, in conjunction with publicly available sequences, representing avipoxvirus strains from six continents 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. These results are novel in their characterization and comparison of the phylogenetic relationships of poxvirus isolates in wild bird species from North America. Further, we provide new data on the level of host specificity and specific strains circulating in North America.

Murray, M.H., E.W. Lankau, A.D. Kidd, C.N. Welch, T. Ellison, H.C. Adams, E.K. Lipp, and S.M. Hernandez. 2020. Gut microbiome shifts with urbanization and potentially facilitates a zoonotic pathogen in a wading bird. *PLoS One* 15(3): e0220926.

ABSTRACT: Microbial communities in the gastrointestinal tract influence many aspects of host health, including metabolism and susceptibility to pathogen colonization. These relationships and the environmental and individual factors that drive them are relatively unexplored for free-living wildlife. We quantified the relationships between urban habitat use, diet, and age with microbiome composition and diversity for 82 American white ibises (*Eudocimus albus*) captured along an urban gradient in south Florida and tested whether gut microbial diversity was associated with *Salmonella enterica* prevalence. Shifts in community composition were significantly associated with urban land cover and, to a lesser extent, diets higher in provisioned food. The diversity of genera was negatively associated with community composition associated with urban land cover, positively associated with age class, and negatively associated with *Salmonella* shedding. Our results suggest that shifts in both habitat use and diet for urban birds significantly alter gut microbial composition and diversity in ways that may influence health and pathogen susceptibility as species adapt to urban habitats.

Niedringhaus, K.D., J.D. Brown, M.A. Ternent, C.A. Cleveland, and M.J. Yabsley. 2019. A serosurvey of multiple pathogens in American black bears (*Ursus americanus*) in Pennsylvania, USA, indicates a lack of association with sarcoptic mange. *Veterinary Science* 6(4): 75.

ABSTRACT: Infectious diseases, particularly of wildlife, are intrinsically linked to human and domestic animal health. Reports of sarcoptic mange in black bears (*Ursus americanus*) are increasing in multiple states in the USA and while the reason is unknown, mange in other species has been associated with immunosuppression from multiple causes. Serum from bears across Pennsylvania were collected to determine the seroprevalence of five pathogens important for animal and/or human health: Canine distemper virus (CDV), canine parvovirus (CPV), canine adenovirus-1 (CAV), *Toxoplasma gondii*, and *Trichinella* sp. from bears with sarcoptic mange as well as bears that were clinically normal. Several of these pathogens, particularly canine distemper virus, are associated with immunosuppression and secondary infections in other hosts. In addition to describing the seroprevalence and relating these findings to data from other regions, statistics were performed to determine if antibodies to any of these pathogens were associated with mange in bears. The overall seroprevalence to these pathogens was as follows: CDV 7.1% (17/240), CPV 16% (15/94), CAV 6.9% (6/87), *Toxoplasma gondii* 64.9% (194/299), and *Trichinella spiralis* 3.2% (7/220). While there was no association between mange and antibodies to these pathogens, infection with one or more of these pathogens has implications for bears, other wildlife, domestic animal, and human health.

Niedringhaus, K.D., J.D. Brown, M.A. Ternent, S.K. Peltier, P. Van Wick, and M.J. Yabsley. 2020. Serology as a tool to investigate sarcoptic mange in American black bears (*Ursus americanus*). *Journal of Wildlife Diseases* 56(2): 350-358.

ABSTRACT: Black bears (*Ursus americanus*) have historically been considered an uncommon host for sarcoptic mange. However, over the last 25 yr, sarcoptic mange has been increasingly reported in black bears in the northeastern U.S.. Syndromic monitoring is the most common surveillance approach for mange in bears, but tools to monitor exposure to *Sarcoptes scabiei* in bear populations have not been thoroughly evaluated under field conditions. In this study, we validated a commercially available enzyme-linked immunosorbent assay (ELISA), designed to detect antibodies against *S. scabiei* in dogs, for use in black bears with a sensitivity and specificity of 95.6% and 96.6%, respectively. To further examine the performance of this assay, serial serum samples from seven black bears with confirmed sarcoptic mange were collected posttreatment to determine the persistence of detectable antibody response with the ELISA. Antibodies in black bears waned to below the limit of detection between 4 and 14 wk, suggesting that serology studies might underestimate the number of exposed black bears after antibodies have waned. State-wide serosurveys in Pennsylvania from hunter-harvested black bears in 2017 and 2018 showed a significant difference in seroprevalence between regions with high occurrence of mange (mean seroprevalence 6.7%, range of 6.6-6.8%) and low occurrence of mange (no seropositive black bears were detected). Within Pennsylvania, these data indicate that the geographic distribution of exposure to *S. scabiei*, based on serologic testing, generally reflects the distribution of overt disease, as determined by syndromic surveillance. Collectively, these results indicate the evaluated ELISA is an effective tool for monitoring *S. scabiei* exposure in bear populations and provides the framework for additional studies regarding sarcoptic mange epidemiology in black bears.

Niedringhaus, K.D., J.D. Brown, M.A. Ternent, S.K. Peltier, and M.J. Yabsley. 2019. Effects of temperature on the survival of *Sarcoptes scabiei* of black bear (*Ursus americanus*) origin. *Parasitology Research* 118(10): 2767-2772.

ABSTRACT: For two decades, the incidence and range of sarcoptic mange in black bears (*Ursus americanus*) in Pennsylvania has increased. The causative agent, *Sarcoptes scabiei*, can be directly or indirectly transmitted; therefore, data on environmental persistence is important for guiding management and public communications. The objective of this study was to determine the survival of *S. scabiei* at different temperatures. Full section skin samples and superficial skin scrapes were collected from bears immediately after euthanasia due to severe mange. After ~ 24 h on ice packs (shipment to lab), samples were placed in dishes at 0, 4, 18, or 30ºC and 60, 20, 12, and 25% relative humidity, respectively, and the percentage of mites alive, by life stage, was periodically determined. Humidity was recorded but not controlled. Temperature significantly affected mite survival, which was shortest at 0°C (mostly ≤ 4 h) and longest at 4ºC (up to 13 days). No mites survived beyond 8 days at 18ºC or 6 days at 30ºC. Mites from full-thickness skin sections survived significantly longer than those from superficial skin scrapes. Adults typically survived longer than nymphs and larvae except at 30ºC where adults survived the shortest time. These data indicate that at cooler temperatures, *S. scabiei* can survive for days to over a week in the environment, especially if on host skin. However, these data also indicate that the environment is unlikely to be a long-term source of *S. scabiei* infection to bears, other wildlife, or domestic animals.

Niedringhaus, K.D., N.M. Nemeth, H.S. Sellers, J.D. Brown, and H.M.A. Fenton. 2019. Multicentric round cell neoplasms and their viral associations in wild turkeys (*Meleagris gallopavo*) in the Southeastern United States. *Veterinary Pathology* 56(6): 915-920.

ABSTRACT: Multiple oncogenic viruses, including lymphoproliferative disease virus (LPDV) and reticuloendotheliosis virus (REV), have been detected in wild turkeys (*Meleagris gallopavo*). The prevalence of infection with these viruses appears to be more common than overt disease; thus, data on the manifestation of associated disease in wild turkeys are scarce. Diagnostic records from wild turkeys submitted to the Southeastern Cooperative Wildlife Disease Study from 1980 to 2017 were reviewed to identify cases of neoplasia. Neoplasia was reported in 59 of 851 (6.9%) wild turkeys submitted. Of the cases of neoplasia tested by polymerase chain reaction, LPDV was detected in 34 of 58 (59%), REV in 10 of 39 (26%), both viruses in 3 of 39 (8%), and no retroviruses detected in 5 of 39 (13%) turkeys. The most common gross lesions observed among turkeys with neoplasms were emaciation (30/40; 75%); nodules in the skin (26/59; 44%), liver (17/59; 29%), or spleen (9/59; 15%); and splenomegaly (14/59; 24%). Microscopically, nodules were composed of pleomorphic round cells with large eccentric nuclei and prominent nucleoli resembling lymphocytes or lymphoblasts (57/59; 97%) except for 2 cases, one of myeloid cell origin and the other with primarily spindloid cells. This study indicates the need to characterize the pathogenesis and potential health threat posed by REV and LPDV to wild turkeys. Experimental infection studies and the development of additional diagnostic tests to confirm the role of retroviruses in lymphoproliferative disease are warranted.

Oakes, V.J., M.J. Yabsley, D. Schwartz, T. LeRoith, C. Bissett, C. Broaddus, J.L. Schlater, S.M. Todd, K.M. Boes, M. Brookhart, and K.K. Lahmers. 2019. *Theileria orientalis* Ikeda genotype in cattle, Virginia USA. *Emerging Infectious Diseases* 25(9): 1653-1659.

ABSTRACT: *Theileria orientalis* Ikeda genotype is a parasite that causes a disease in cattle that results in major economic issues in Asia, New Zealand, and Australia. The parasite is transmitted by *Haemaphysalis longicornis* ticks, which have recently been reported in numerous states throughout the eastern United States. Concurrently, cattle in Virginia showed clinical signs consistent with a hemoprotozoan infection. We used amplicons specific for the major piroplasm surface protein and small subunit rDNA of piroplasms to test blood samples from the cattle by PCR. Bidirectional Sanger sequencing showed sequences with 100% identity with *T. orientalis* Ikeda genotype 2 sequences. We detected the parasite in 3 unrelated herds and from various animals sampled at 2 time points. Although other benign *T. orientalis* genotypes are endemic to the United States, detection of *T. orientalis* Ikeda genotype might represent a risk for the cattle industry in Virginia.

Poulson, R.L., and J.D. Brown. 2020. Wild bird surveillance for avian influenza virus. *Methods Mol Biol.* 2020;2123:93-112. doi: 10.1007/978-1-0716-0346-8_8.

ABSTRACT: Avian influenza (AI) viruses have been routinely isolated from a wide diversity of free-living avian species, representing numerous taxonomic orders. Birds in orders Anseriformes and Charadriiformes are considered the natural reservoirs for all AI viruses; it is from these orders that AI viruses have been most frequently isolated. Since first recognized in the late 1800s, AI viruses have been an important cause of disease in poultry and, occasionally, in non-gallinaceous birds and mammals. While AI viruses tend to be of low pathogenicity (LP) in wild birds, the 2014-2015 incursion of highly pathogenic avian influenza (HPAI) clade 2.3.4.4 H5Nx viruses into North America and the recent circulation of HPAI H5 viruses in European wild birds highlight the need for targeted, thorough, and continuous surveillance programs in the wild bird reservoir. Such programs are crucial to understanding the potential risk for the incursion of AI into human and domestic animal populations. The aim of this chapter is to provide general concepts and guidelines for the planning and implementation of surveillance plans for AI viruses in wild birds.

Reeves, A.B., A.M. Ramey, J.C. Koch, R.L. Poulson, and D.E. Stallknecht. 2020. Field-based method for assessing duration of infectivity for influenza A viruses in the environment. *Journal of Virology Methods* doi: 10.1016/j.jviromet.2020.113818.

ABSTRACT: Understanding influenza A virus (IAV) persistence in wetlands is limited by a paucity of field studies relating to the maintenance of infectivity over time. The duration of IAV infectivity in water has been assessed under variable laboratory conditions, but results are difficult to translate to more complex field conditions. We tested a field-based method to assess the viability of IAVs in an Alaska wetland during fall and winter which incorporated physical and chemical properties of the waterbody in which samples were held. Filtered pond water was inoculated with avian fecal samples collected from the environment, aliquoted into a series of duplicate sealed vials and submerged back in the wetland for up to 132 days (October 2018-March 2019). Sample aliquots were sequentially recovered and tested for IAVs by rRT-PCR and virus isolation. One sample remained rRT-PCR positive for the duration of the study and virus isolation positive for 118 days. The surrounding water temperature was 1°-6 °C with near neutral pH (6.6–- for the duration of the study. This proof of concept study demonstrates a protocol for testing the persistence of infectious IAV naturally shed from waterfowl under ambient environmental conditions.

Rothenburger, J.L., C.G. Himsworth, N.M. Nemeth, D.L. Pearl, P.M. Treuting, and C.M. Jardine. 2019. The devil is in the details-Host disease and co-infections are associated with zoonotic pathogen carriage in Norway rats (*Rattus norvegicus*). *Zoonoses and Public Health* 66(6): 622-635.

ABSTRACT: Traditionally, zoonotic pathogen ecology studies in wildlife have focused on the interplay among hosts, their demographic characteristics and their pathogens. But pathogen ecology is also influenced by factors that traverse the hierarchical scale of biological organization, ranging from within-host factors at the molecular, cellular and organ levels, all the way to the host population within a larger environment. The influence of host disease and co-infections on zoonotic pathogen carriage in hosts is important because these factors may be key to a more holistic understanding of pathogen ecology in wildlife hosts, which are a major source of emerging infectious diseases in humans. Using wild Norway rats (*Rattus norvegicus*) as a model species, the purpose of this study was to investigate how host disease and co-infections impact the carriage of zoonotic pathogens. Following a systematic trap and removal study, we tested the rats for the presence of two potentially zoonotic bacterial pathogens (*Bartonella tribocorum* and *Leptospira interrogans*) and assessed them for host disease not attributable to these bacteria (i.e., nematode parasites, and macroscopic and microscopic lesions). We fitted multilevel multivariable logistic regression models with pathogen status as the outcome, lesions and parasites as predictor variables and city block as a random effect. Rats had significantly increased odds of being infected with *B. trib*ocorum if they had a concurrent nematode infection in one or more organ systems. Rats with bite wounds, any macroscopic lesion, cardiomyopathy or tracheitis had significantly increased odds of being infected with *L. interrogans*. These results suggest that host disease may have an important role in the ecology and epidemiology of rat-associated zoonotic pathogens. Our multiscale approach to assessing complex intrahost factors in relation to zoonotic pathogen carriage may be applicable to future studies in rats and other wildlife hosts.

Self, S.C., Y. Liu, S.K. Nordone, M.J. Yabsley, H.S. Walden, R.B. Lund, D.D. Bowman, C. Carpenter, C.S. McMahan, and J.R. Gettings. 2019. Canine vector-borne disease: mapping and the accuracy of forecasting using big data from the veterinary community. *Animal Health Research Reviews* 20(1): 47-60.

ABSTRACT: Diagnosis, treatment, and prevention of vector-borne disease (VBD) in pets is one cornerstone of companion animal practices. Veterinarians are facing new challenges associated with the emergence, reemergence, and rising incidence of VBD, including heartworm disease, Lyme disease, anaplasmosis, and ehrlichiosis. Increases in the observed prevalence of these diseases have been attributed to a multitude of factors, including diagnostic tests with improved sensitivity, expanded annual testing practices, climatologic and ecological changes enhancing vector survival and expansion, emergence or recognition of novel pathogens, and increased movement of pets as travel companions. Veterinarians have the additional responsibility of providing information about zoonotic pathogen transmission from pets, especially to vulnerable human populations: the immunocompromised, children, and the elderly. Hindering efforts to protect pets and people is the dynamic and ever-changing nature of VBD prevalence and distribution. To address this deficit in understanding, the Companion Animal Parasite Council (CAPC) began efforts to annually forecast VBD prevalence in 2011. These forecasts provide veterinarians and pet owners with expected disease prevalence in advance of potential changes. This review summarizes the fidelity of VBD forecasts and illustrates the practical use of CAPC pathogen prevalence maps and forecast data in the practice of veterinary medicine and client education.

Stallknecht, D.E., C. Kienzle-Dean, N. Davis-Fields, C.S. Jennelle, A.S. Bowman, J.M. Nolting, W.M. Boyce, J.M. Crum, J.J.S. Santos, J.D. Brown, D.J. Prosser, S.E.W. De La Cruz, J.T. Ackerman, M.L. Casazza, S. Krauss, D.R. Perez, A.M. Ramey, and R.L. Poulson. 2020. Limited detection of antibodies to clade 2.3.4.4 A/goose/Guangdong/1/1996 lineage highly pathogenic H5 avian influenza virus in North American waterfowl. *Journal of Wildlife Diseases* 56(1): 47-57.

ABSTRACT: During 2014, highly pathogenic (HP) influenza A viruses (IAVs) of the A/Goose/Guangdong/1/1996 lineage (GsGD-HP-H5), originating from Asia, were detected in domestic poultry and wild birds in Canada and the US. These clade 2.3.4.4 GsGD-HP-H5 viruses included reassortants possessing North American lineage gene segments; were detected in wild birds in the Pacific, Central, and Mississippi flyways; and caused the largest HP IAV outbreak in poultry in US history. To determine if an antibody response indicative of previous infection with clade 2.3.4.4 GsGD-HP-H5 IAV could be detected in North American wild waterfowl sampled before, during, and after the 2014-15 outbreak, sera from 2,793 geese and 3,715 ducks were tested by blocking enzyme-linked immunosorbent assay and hemagglutination inhibition (HI) tests using both clade 2.3.4.4 GsGD-HPH5 and North American lineage low pathogenic (LP) H5 IAV antigens. We detected an antibody response meeting a comparative titer-based criteria (HI titer observed with 2.3.4.4 GsGD-HP-H5 antigens exceeded the titer observed for LP H5 antigen by two or more dilutions) for previous infection with clade 2.3.4.4 GsGD-HP-H5 IAV in only five birds, one blue-winged teal (*Spatula discors*) sampled during the outbreak and three mallards (*Anas platyrhynchos*) and one Canada goose (*Branta canadensis*) sampled during the post-outbreak period. These serologic results are consistent with the spatiotemporal extent of the outbreak in wild birds in North America during 2014 and 2015 and limited exposure of waterfowl to GsGD-HP-H5 IAV, particularly in the central and eastern U.S.

Swenson, S.L., D.G. Mead, and D.R. Kinker. 2019. Rhabdoviruses; rabies virus and vesicular stomatitis viruses. In: Diseases of Swine, 11th Edition. pp. 733-739. J.J. Zimmerman, L.A. Karriker, A. Ramirez, K.J. Schwartz, G.W. Stevenson, and J. Zhang (eds). Wiley-Blackwell Publishing, Ames, IA.

Taylor, K.Y., M.G. Ruder, D.G. Mead, and D.E. Stallknecht. 2020. An embryonated egg transmission model for epizootic hemorrhagic disease virus. *Vector-Borne and Zoonotic Diseases* doi: 10.1089/vbz.2019.2590.

ABSTRACT: Epizootic hemorrhagic disease virus (EHDV) is a vector-borne orbivirus of ruminants; in North America there are three serotypes (EHDV-1, -2, and -6) and these primarily affect white-tailed deer (*Odocoilus virginianus*). EHDV is vectored by biting midges, *Culicoides* spp. Embryonated chicken eggs (ECE) have recently been used as an experimental host to investigate the vector competence of Australian *Culicoides* spp. for bluetongue serotype virus 1 and 23. In this study, we evaluated the use of the ECE model to determine its applicability for evaluating vector competence related to transmission of North American EHDV serotypes. We demonstrated that all three North American EHDV serotypes were able to replicate in ECEs and be transmitted from infected ECEs to *Culicoides sonorensis* Wirth & Jones. In addition, we were able to complete the transmission cycle from infected *C. sonorensis* to uninfected ECEs for EHDV-1 and -2.

Thompson, A.T., C.A. Cleveland, T.M. Koser, S.T. Wyckoff, and M.J. Yabsley. 2019. The occurrence of *Physaloptera hispida* and a *Mastophorus* sp. in pulmonary vessels of hispid cotton rats (*Sigmodon hispidus*) from Georgia, USA. *Journal of Parasitology* 105(5): 718-723.

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 was conducted. On 4 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 3 as a *Mastophorus* sp. by morphology. No *A. cantonensis* were found. *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. 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.

Thompson, A.T., K. Dominguez, C.A. Cleveland, S.J. Dergousoff, K. Doi, R.C. Falco, T. Greay, P. Irwin, L. Robbin Lindsay, J. Liu, T.N. Mather, C. Oskam, R.I. Rodriquez-Vivas, M.G. Ruder, D. Shaw, S.L. Vigil, S. White, and M.J. Yabsley. 2020. Molecular characterization of *Haemaphysalis* species and a molecular genetic key for the identification of *Haemaphysalis* of North America. *Frontiers in Veterinary Science* 7:141; doi: 10.3389/fvets.2020.00141.

ABSTRACT: *Haemaphysalis longicornis* (Acari: Ixodidae), the Asian longhorned tick, is native to East Asia, but has become established in Australia and New Zealand, and more recently in the United States. In North America, there are other native *Haemaphysalis* species that share similar morphological characteristics and can be difficult to identify if the specimen is damaged. The goal of this study was to develop a cost-effective and rapid molecular diagnostic assay to differentiate between exotic and native *Haemaphysalis* species to aid in ongoing surveillance of *H. longicornis* within the United States and help prevent misidentification. We demonstrated that restriction fragment length polymorphisms (RFLPs) targeting the 16S ribosomal RNA and the cytochrome *c* oxidase subunit I (*COI*) can be used to differentiate *H. longicornis* from the other *Haemaphysalis* species found in North America. Furthermore, we show that this RFLP assay can be applied to *Haemaphysalis* species endemic to other regions of the world for the rapid identification of damaged specimens. The work presented in this study can serve as the foundation for region specific PCR-RFLP keys for *Haemaphysalis* and other tick species and can be further applied to other morphometrically challenging taxa.

Thompson, A.T., S. White, D. Shaw, A. Egizi, K. Lahmers, M.G. Ruder, and M.J. Yabsley. 2020. Theileria *orientalis* Ikeda in host-seeking *Haemaphysalis longicornis* in Virginia, U.S.A. *Ticks and Tick-Borne Diseases* 11 101450; doi.org/10.1016/j.ttbdis.2020.101450.

ABSTRACT: The Asian longhorned tick, *Haemaphysalis longicornis*, has recently become established in the United States. In East Asia, Australia, and New Zealand, the native and previously introduced ranges, this tick is a vector of an important pathogen of cattle, *Theileria orientalis*. In 2017, the pathogenic Ikeda genotype of *T. orientalis* was associated with cattle mortalities in Virginia and in 2018 the exotic *H. longicornis* was detected at this same site. To investigate the possible role of this exotic tick in the epidemiology of theileriosis in Virginia, we tested host-seeking *H. longicornis* for piroplasm infections. We document the detection of exotic *Theileria orientalis* Ikeda genotype in 12.7% (15/118) environmentally collected *H. longicornis* using both the 18S rRNA and major piroplasm surface protein (*MPSP*) gene targets. This is the first detection of a pathogen in *H. longicornis* in its introduced range in the United States and offers new insight into the animal health risks associated with the introduction of this exotic tick species to North America.

Verhagen, J.H., M. Poen, D.E. Stallknecht, S. van der Vliet, P. Lexmond, S. Sreevatsan, R.L. Poulson, R.A. Fouchier, and C. Lebarbenchon. 2020. Phylogeography and antigenic diversity of low pathogenic avian influenza H13 and H16 viruses. *Journal of Virology* doi: 10.1128/JVI.00537.20.

ABSTRACT: Low-pathogenic avian influenza viruses (LPAIVs) are genetically highly variable and have diversified into multiple evolutionary lineages that are primarily associated with wild-bird reservoirs. Antigenic variation has been described for mammalian influenza viruses and for highly pathogenic avian influenza viruses that circulate in poultry, but much less is known about antigenic variation of LPAIVs. In this study, we focused on H13 and H16 LPAIVs that circulate globally in gulls. We investigated the evolutionary history and intercontinental gene flow based on the hemagglutinin (HA) gene and used representative viruses from genetically distinct lineages to determine their antigenic properties by hemagglutination inhibition assays. For H13, at least three distinct genetic clades were evident, while for H16, at least two distinct genetic clades were evident. Twenty and ten events of intercontinental gene flow were identified for H13 and H16 viruses, respectively. At least two antigenic variants of H13 and at least one antigenic variant of H16 were identified. Amino acid positions in the HA protein that may be involved in the antigenic variation were inferred, and some of the positions were located near the receptor binding site of the HA protein, as they are in the HA protein of mammalian influenza A viruses. These findings suggest independent circulation of H13 and H16 subtypes in gull populations, as antigenic patterns do not overlap, and they contribute to the understanding of the genetic and antigenic variation of LPAIVs naturally circulating in wild birds. Wild birds play a major role in the epidemiology of low-pathogenic avian influenza viruses (LPAIVs), which are occasionally transmitted—directly or indirectly—from them to other species, including domestic animals, wild mammals, and humans, where they can cause subclinical to fatal disease. Despite a multitude of genetic studies, the antigenic variation of LPAIVs in wild birds is poorly understood. Here, we investigated the evolutionary history, intercontinental gene flow, and antigenic variation among H13 and H16 LPAIVs. The circulation of subtypes H13 and H16 seems to be maintained by a narrower host range, in particular gulls, than the majority of LPAIV subtypes and may therefore serve as a model for evolution and epidemiology of H1 to H12 LPAIVs in wild birds. The findings suggest that H13 and H16 LPAIVs circulate independently of each other and emphasize the need to investigate within-clade antigenic variation of LPAIVs in wild birds.

Vincent, E.C., M.G. Ruder, M.J. Yabsley, V.S. Hesting, M.K. Keel, J.D. Brown, and N.M. Nemeth. 2020. A *Baylisascaris* outbreak in fox squirrels (*Sciurus niger)* and subsequent detection of *Francisella tularensis* in Kansas, USA. *Journal of Wildlife Diseases* 56(2): 457-461.

ABSTRACT: Thorough epidemiologic investigations of wildlife mortality events are often challenging, in part because of the dynamic variables involved. In May 2011, six fox squirrels (*Sciurus niger*) in Clinton State Park, Kansas, US were euthanized after exhibiting clinical signs of neurologic disease. Postmortem examination of two squirrels revealed that these individuals died of *Baylisascaris* larva migrans, which resulted in meningoencephalitis and variable pneumonia and myocarditis. Fecal flotation of raccoon (*Procyon lotor*) feces collected in the area revealed *Baylisascaris* sp. ova, presumably *Baylisascaris procyonis*, in one of nine samples. Additional fox squirrel carcasses were submitted for diagnostic evaluation from eastern Kansas for 1 yr following the *Baylisascaris* sp. outbreak. This monitoring unexpectedly resulted in the detection of *Francisella tularensis*, the zoonotic pathogen that causes tularemia, in two fox squirrels. The increased attention to fox squirrel mortalities prompted by the outbreak of *Baylisascaris* sp. larva migrans revealed cases of tularemia that may not have been otherwise detected. Although *F. tularensis* is endemic in Kansas, the current distribution and prevalence of *B. procyonis* in raccoons and other hosts in Kansas are poorly understood. This yearlong mortality investigation illustrated the importance of wildlife health monitoring as a means of assessing public health risks, especially during unusual wildlife mortality events.

White, S.A., S.N. Bevins, M.G. Ruder, D. Shaw, S.L. Vigil, A. Randall, T. Deliberto, K. Dominquez, A.T. Thompson, J.W. Mertins, J. Alfred, and M.J. Yabsley. 2020. Surveys for ticks on wildlife hosts and in the environment at Asian longhorned tick (*Haemaphysalis longicornis*)-positive sites in Virginia and New Jersey, 2018. *Transboundary and Emerging Diseases* https://doi.org/10.1111/tbed.13722.

ABSTRACT: *Haemaphysalis longicornis* , the Asian longhorned tick (ALT), is native to eastern Asia, but it has become invasive in several countries, including Australia, New Zealand and recently in the eastern United States (U.S.). To identify wild mammal and avian host species in the US, we conducted active wildlife surveillance in two states with known ALT infestations (Virginia and New Jersey). In addition, we conducted environmental surveys in both states. These surveillance efforts resulted in detection of 51 ALT-infested individuals from seven wildlife species, including raccoon (*Procyon lotor*), Virginia opossum (*Didelphis virginiana*), red fox (*Vulpes vulpes*), woodchuck (*Marmota monax*), eastern cottontail (*Sylvilagus floridanus*), striped skunk (*Mephitis mephitis*) and white-tailed deer (*Odocoileus virginianus*). We found ALT in the environment in both states and also collected three native tick species (*Amblyomma americanum, Dermacentor variablis* and *Ixodes scapularis*) that are vectors of pathogens of public health and veterinary importance. This study provides important specific information on the wildlife host range of ALT in the US.

In Press/Accepted to Journal:

Allen, S.E., C.M. Jardine, K. Hooper-McGrevy, A. Ambagala, A.M. Bosco-Lauth, M.R. Kunkel, D.G. Mead, L. Nituch, M.G. Ruder, and N.M. Nemeth. Serological evidence of arthropod-

borne virus infections in wild and captive ruminants in Ontario, Canada. *The American Journal of Tropical Medicine and Hygiene*.

Cleveland, C.A., L. Swanepoel, J.D. Brown, M.J. Casalena, L. Williams, and M.J. Yabsley. Surveillance for *Borrelia* spp. in upland game birds from Pennsylvania, USA. *Ticks and Tick-Borne* Diseases.

Keel, M.K., S. Keeler, J. Brown, H. Fenton, B. Munk, R. Gerhold, N. Gottdenker, M. Ruder, and N. Nemeth. Granulomatous inflammation of the muzzle in white-tailed deer (*Odocoileus virginianus*) and mule deer (*O. hemionus*) associated with *Mannheimia granulomatis*. *Veterinary Pathology.*

McKenzie, C., P. Oesterie, B. Stevens, L. Shirose, B. Lillie, C. Davy, C. Jardine, and N. Nemeth. Pathology associated with ophidiomycosis in wild snakes in Ontario, Canada. *Canadian Veterinary Journal*.

Sapp, S.E.G., and M.J. Yabsley. Experimental comparison of *Baylisascaris procyonis* definitive host competence between domestic dogs and raccoons (*Procyon lotor*). *Parasitology.*

Willis, E., K. Niedringhaus, J. Ballard, and N. Nemeth. Pathology in Practice: Chondrosarcome in a duck. *Journal of the American Veterinary Medical Association*.

EXTENSION AND OTHER PUBLIC SERVICE ACTIVITIES

Committees

Members of the SCWDS professional staff collectively have served in a wide variety of assignments for professional associations and work-related organizations. The professional service work, although demanding in time and expense, has proved valuable in maintaining liaison among State and Federal Fish and Wildlife Agencies, State and Federal Agricultural and Public Health Agencies, private citizens, and private organizations interested in wildlife and the livestock and poultry industries.

Dr. Sonia M. Hernandez: Chair, Wildlife Veterinary Section of Wildlife Disease Association; Strategic Planning Committee of Wildlife Disease Association; Chair of One Health Program at the Veterinary Medical Expo (NAVC); Program Chair Coordinator, NAVC; Stoddard-Dutton Conservation/Ornithology Award Committee, UGA; Member, Admissions Committee, College of Veterinary Medicine.

Dr. Daniel Mead: Member, Committee on Wildlife, U.S. Animal Health Association (USAHA); Member, Committee on Parasitic and Vector Borne Diseases, USAHA; Member, West Nile Virus Working Group, State of Georgia; Member, DVM-MPH Committee, College of Veterinary Medicine (CVM), The University of Georgia (UGA); Chair, AHRC Oversight Committee, CVM, UGA; Member, Biosafety Community Liaison Committee, CVM, UGA; Member, FID Advisory Committee, Office of the Vice President for Research, UGA; Member, University Council, Office of the President, UGA.

Dr. Nicole Nemeth: Member, National White Nose Syndrome Diagnostic Working Group; Member, National Bsal Task Force Technical Advisory Committee; Committee Member, Animal Health Research Center, UGA; Committee Member, Staff Appreciation Awards Committee member, UGA; Participant, Recruitment and Selection, Anatomic Pathology Residency, UGA.

She also served as an assessor and student mentor for the Specialty Focus area of emphasis (zoo/wildlife) for the DVM Class of 2022 (April 2020) as well as an application reviewer and mentor for the Georgia Veterinary Scholars Program.

Dr. Mark Ruder: Co-Chair, Southeastern Wildlife Health Technical Group; Vice-Chair, Committee on Wildlife, USAHA; Member, Parasitic and Vector-Borne Diseases, USAHA; Member, Committee on Farmed Cervidae, USAHA; Member, Fish and Wildlife Health Committee, Association of Fish and Wildlife Agencies; Advisory Board, American Association of Wildlife Veterinarians; Government Relations Committee, American Association of Wildlife Veterinarians.

Dr. David Stallknecht: Editorial Board, *Journal of Wildlife Diseases*, Animal Resources Committee and Covid-19 Task Force, College of Veterinary Medicine, and the AFWA Wildlife Health Committee.

Dr. Michael Yabsley: Member, UGA Promotion and Tenure Appeals Committee (CVM representative); Member, UGA Promotion and Tenure Review Committee- Applied/Clinical Sciences D section; Member, UGA Institutional Animal Care and Use Committee Alternate; Member, IDEAS (Infectious Disease Ecology Across Scales) Steering Committee; Member, IDEAS (Infectious Disease Ecology Across Scales) Admissions Committee; Member, Research Awards Review Committee, Warnell; Member, Board of the Companion Animal Parasite Council.

TRAVEL ASSOCIATED WITH SCWDS ACTIVITIES

July 6-11, 2019
Stallknecht. Lansing, MI. To attend the National Fish and Wildlife Strategic Planning Meeting.

July 7-11, 2019
Gettings and Thompson. Madison, WI. To attend and present research at the 27th International World Association for the Advancement of Veterinary Parasitology. Mr. Thompson gave a presentation entitled "Development of a Diagnostic Assay for Detection and Differentiation of *Theileria* spp. on White-tailed Deer."

July 8-10, 2019
Ruder. Kingsville, TX. To attend the 2019 Southeast Deer Technical Committee meeting and give SCWDS updates.

July 8-21, 2019
Shaw and White. Waynesboro, VA. To conduct exotic arthropod surveillance using environmental and wildlife sampling.

August 2-12, 2019
Dugovich. Lake Tahoe, CA. To attend the 68th Wildlife Disease Association Annual International Conference, and give a presentation entitled "Foot and Mouth Disease Epidemiology in a Herd of Its Natural Reservoir Host, African Buffalo."

August 3-9, 2019
Stallknecht. Lake Tahoe, CA. To attend the 68th Wildlife Disease Association Annual International Conference and the Editorial Board Meeting for the *Journal of Wildlife Diseases*.

August 4-9, 2019
Ruder and Thompson. Lake Tahoe, CA. To attend the 68th Wildlife Disease Association Annual International Conference. Dr. Ruder to give a presentation entitled "Anticoagulant Rodenticide Exposure in Bald Eagles (*Haliaeetus leucocephalus*) and Golden Eagles (*Aquila chrysaetos*) in the Unites States," and attend a separate meeting between USGS and SCWDS.

August 12-14, 2019
Fischer and Ruder. Starkville, MS. To attend and present at the Chronic Wasting Disease Meeting.

August 22-September 10, 2019
Cleveland. Ndjamena, Chad. To conduct field work to check game cameras for wildlife surveillance in support of the Guinea worm research.

August 23-September 23, 2019
Fojtik. Middle River, MN. To collect samples from waterfowl for the avian influenza virus surveillance project.

September 3-5, 2019
White and Wlodkowski. Romney, WV. To conduct herd health evaluations with the West Virginia Division of Natural Resources in Hampshire and Hardy counties.

September 5-11, 2019
Thompson and White. Waynesboro, VA. To collect samples from wildlife for exotic arthropod surveillance.

September 11-28, 2019
Carter. Winnie, TX and Baton Rouge, LA. To collect oral and fecal samples from blue-winged teal during the fall migration for the avian influenza virus surveillance project.

September 22-25, 2019
Stallknecht. St. Paul, MN. To attend the 109th Association of Fish and Wildlife Agencies (AFWA) Annual Meeting.

September 29-October 3, 2019
Cleveland and Williams. Reno, NV. To attend the 2019 The Wildlife Society Conference. Dr. Cleveland to give presentations entitled "*Dracunculus insignis* in Wildlife in Georgia" and "*Borrelia* in Upland Game Birds in Pennsylvania," and Ms. Williams to give a presentation entitled "Surveillance for Influenza A Viruses in a Breeding Seabird Colony on Middleton Island, Alaska."

October 6-19, 2019
Wyckoff. Arnett, Frederick, Lawton, Norman, Oklahoma City, Stillwater, Turpin, and Woodward, OK. To conduct northern bobwhite health surveillance at various sites in Oklahoma.

October 20-21, 2019
Cleveland. Knoxville, TN. To give a presentation entitled "The Wild World of Guinea Worms" at a University of Tennessee-Knoxville seminar.

October 27-30, 2019
Ruder. Providence, RI. To attend the 2019 U.S. Animal Health Association Annual Meeting (USAHA) and give presentations entitled "SCWDS Update – EHDV/BTV Surveillance and Tick

Projects" and "2019 Hemorrhagic Disease Update and Asian Longhorned Tick Surveillance" to the Committee on Wildlife and the Committee on Parasitic and Vector-Borne Diseases.

<u>October 27-30, 2019</u>
Stallknecht. Hilton Head, SC. To attend the 73rd annual Southeastern Association of Fish and Wildlife Agencies (SEAFWA) Conference.

<u>October 29-November 15, 2019</u>
Cleveland. N'Djamena, Chad, Africa. To begin round two of flubendazole treatments in domestic dogs for Guinea worm and retrieve game cameras.

<u>November 4, 2019</u>
Ruder and Stallknecht. Columbia, SC. To meet with the South Carolina Department of Natural Resources concerning CWD urine ban.

<u>November 4-8, 2019</u>
Vigil. Fort Collins, CO. To attend the annual meeting of collaborators from SCWDS, USDA-APHIS-VS, and University of Melbourne on the AADIS Cooperative Agreement funded by USDA.

<u>November 8, 2019</u>
Ruder. Aikens, SC. To give a presentation the University of South Carolina-Aikens, titled "Understanding hemorrhagic disease of deer".

<u>November 9-16, 2019</u>
Nemeth. San Antonio, TX. To attend the annual meeting of the American College of Veterinary Pathologists and give a presentation entitled "Mortality Event Involving a Novel Bacterial (Family Pasteurellaceae) in Terns, March Island, Florida, USA," learn about latest pathology research and diagnostic technology, and meet potential candidate pathology residents and graduate students.

<u>November 16-21, 2019</u>
Vigil. St. Louis, MO. To attend the Entomological Society of American 2019 Annual Conference and give a presentation entitled "Surveillance of *Culicoides* spp. (Diptera: Ceratophogonidae) at Epizootic Hemorrhagic Disease Outbreak Locations in Michigan."

<u>December 5-17, 2019</u>
White. Edinburg, TX. To conduct cattle fever tick surveillance.

<u>December 6-10, 2019</u>
Fojtik. Edinburg, TX. To conduct cattle fever tick surveillance.

<u>December 16-20, 2019</u>
Carter and Fojtik. Dyersburg, TN. To work with the University of Tennessee in swabbing and bleeding techniques.

<u>January 6-9, 2020</u>
Stallknecht. Madison, WI. To represent SEAFWA and attend a Climate Change and Wildlife Health Workshop.

<u>January 8-21, 2020</u>
White. Edinburg, TX. To conduct an arthropod survey for cattle fever ticks on hunter-harvested animals.

January 20-24, 2020
Carter. Jacksonville, FL and Deveaux Banks, SC. To collect fecal samples from ruddy turnstones for the ongoing avian influenza virus surveillance.

February 1-6, 2020
Fojtik and Kunkel. Piedmont, NC. To assist in the collection of biological samples from wild turkeys.

February 2-7, 2020
Poulson and Stallknecht. Bogota and Santa Marta, Columbia. To attend the Centers of Excellence for Influenza Research and Surveillance Annual Meeting.

February 4-6, 2020
Cleveland. Fort Collins, CO. To attend the USDA-APHIS-WS Feral Swine Stakeholders Meeting.

February 9-12, 2020
Carter. Jacksonville, FL and Deveaux Banks, SC. To collect fecal samples from ruddy turnstones for the ongoing avian influenza virus surveillance.

February 12, 2020
Doub and White. Clayton, GA. To scout locations for exotic arthropod surveillance in Warwoman Wildlife Management Area.

February 15-18, 2020
Cleveland, Stilwell, and Wlodkowski. Jasper, AR. To conduct fieldwork for chronic wasting disease (CWD) research project.

February 15-20, 2020
Ruder. Jasper, AR. To conduct fieldwork for CWD research project and attend the Arkansas Game and Fish Commission Monthly Commission meeting in Little Rock to provide an update on SCWDS activities.

February 23-26, 2020
Stallknecht. Auburn, AL. To attend the Southeastern Deer Study Group Meeting and give a presentation entitled “Changing Hemorrhagic Disease Patterns over Four Decades.”

February 27-March 1, 2020
Stilwell. Nauvoo, AL. To attend the Southeast Partners in Reptile and Amphibian Conservation (SEPARC) Annual Meeting and give a presentation entitled “Amphibian and Reptile Disease Surveillance and Research at the Southeastern Cooperative Wildlife Disease Study.”

February 28, 2020
Cleveland. Atlanta, GA. To meet the new Associate Director Research Guinea Worm Eradication Program at the Carter Center.

March 2-4, 2020
Thompson. Lexington, KY. To attend the One Health Conference and give a presentation entitled “Tracking an Invader: Wildlife and Molecular Surveillance for *Haemaphysalis longicornis* in the Eastern U.S.”

March 3-5, 2020
Ruder. Geary County, KS. To attend the Kansas Department of Wildlife, Parks, and Tourism, Wildlife Division meeting and give a seminar entitled "SCWDS – A Cooperative Approach to Wildlife Health Wildlife Mortality Investigation."

March 3-11, 2020
Cleveland, Ruder, Stillwell, and Wlodkowski. Jackson, TN. To collect samples from white-tailed deer for the CWD Vertical Transmission Study.

March 9-12, 2020
Carter. Walamalaw Island, SC. To collect samples from ruddy turnstones for the avian influenza virus surveillance.

March 22-April 6, 2020
Carter and Fojtik. Baton Rouge, LA and Anahuac, TX. To collect samples from waterfowl for the avian influenza virus surveillance.

March 30-April 1, 2020
Vigil. Atlanta, GA (virtual meeting). To attend the Entomological Society of American Southeastern Branch Annual Conference and give a presentation entitled "Monitoring *Culicoides* spp. (Diptera; Ceratopogonidae) at Two Geographically Similar Locations in the Georgia Piedmont with Differing Land Use Patterns, 2016-2019."

April 7, 2020
White. Tallulah and Black Rock State Park, GA. To conduct fieldwork for *Haemaphysalis longicornis* surveillance.

April 8-10, 2020
Vigil. Jekyll Island, GA (virtual meeting). To attend the Georgia Entomological Society Annual Conference and give a presentation entitled "A Visual Guide to *Culicoides* (Diptera: Ceratophogonidae) Species of the Southeastern United States."

April 14, 2020
White. Tallulah and Black Rock State Park, GA. To conduct fieldwork for *Haemaphysalis longicornis* surveillance.

May 3-9, 2020
Thompson. Waynesboro, VA. To conduct fieldwork for *Haemaphysalis longicornis* surveillance.

May 21-24, 2020
White. Ashmore Heritage Preserve, SC. To conduct fieldwork for *Haemaphysalis longicornis* surveillance.

May 31-June 3, 2020
White. Jocassee Georges Wildlife Management Area, SC To conduct fieldwork for *Haemaphysalis longicornis* surveillance.

June 6-18, 2020
Cleveland. Montpelier, France (virtual meeting). To attend the 2020 Ecology and Evolution of Infectious Diseases International Conference and give a presentation entitled "Hunting Tiny Dragons: Transmission Dynamics, Interventions, and Eradication Challenges of the Guinea Worm (*Dracunculus medinensis*) in Chad, Africa."

June 11-14, 2020
White. Jocassee Gorges Wildlife Management Area, SC To conduct fieldwork for *Haemaphysalis longicornis* surveillance.

June 16-19, 2020
White. Warwoman Wildlife Management Area, GA. To conduct fieldwork for *Haemaphysalis longicornis* surveillance.

June 23-26, 2020
White. Swallow Creek Wildlife Management Area, GA. To conduct fieldwork for *Haemaphysalis longicornis* surveillance.

FINANCIAL STATEMENT FOR FISCAL YEAR 2019-2020

STATE CONTRACT

RF SCWD CONSRTM FISCH – 10-21-RR694-173

CARRY-OVER FROM CONTRACT PERIOD - ENDING JUNE 30, 2019		$466,485.22
NEW CONTRACT FUNDS - JULY 1, 2019 - JUNE 30, 2020		$797,440.00
TOTAL FUNDS AVAILABLE - JULY 1, 2019 - JUNE 30, 2020		$1,263,925.22
EXPENDITURES (7/01/19 - 6/30/20):		
Personal Services and Benefits	$486,215.22	
Operating Expenses	$180,457.72	
Equipment Expenses	$0.00	
Travel	$14,121.09	
TOTAL EXPENDITURES	$680,794.03	$680,794.03
UNENCUMBERED CARRY-OVER INTO CONTRACT PERIOD JULY 1, 2020 - JUNE 30, 2021		$583,131.19