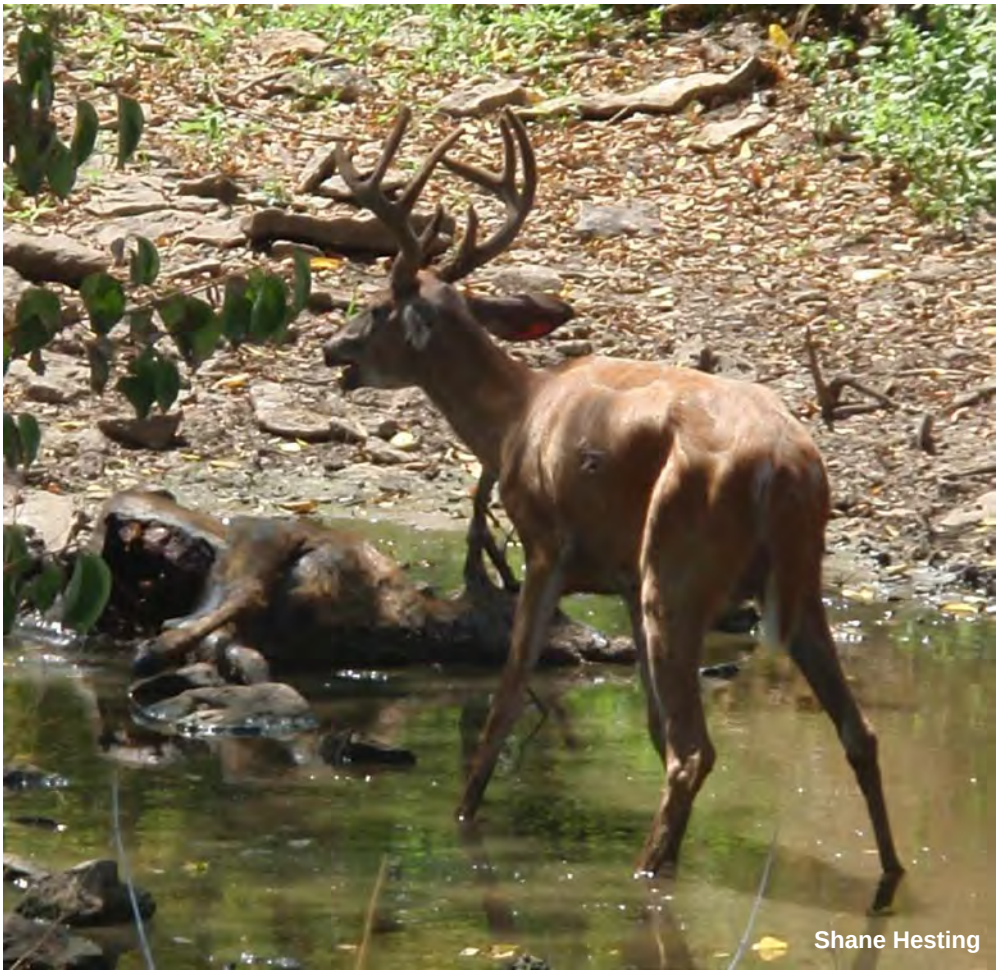
SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY ANNUAL REPORT

July 1, 2012 – June 30, 2013



College of Veterinary Medicine
The University of Georgia
Athens, Georgia

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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 Southeastern Association of Fish and Wildlife Agencies and an appropriation from the Congress of the United States. Funds for the cooperative effort are administered and research is coordinated under the Federal Aid in Wildlife Restoration Act (50 Stat. 917) and through a service grant with the Biological Resources Division of the U.S. Geological Survey, U.S. Department of the Interior. Funding also is provided through Cooperative Agreements 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, New Jersey, North Carolina, Ohio, Oklahoma, Pennsylvania, South Carolina, Tennessee, Virginia, and West Virginia.

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SCWDS STEERING COMMITTEE

Jonathan Gassett, Chairman	Kentucky Department of Fish and Wildlife Resources
David T. Cobb, Vice Chairman	North Carolina Wildlife Resources Commission
Michael Piccirilli, Secretary	U.S. Fish and Wildlife Service
Sheila Allen, Dean	College of Veterinary Medicine, The University of Georgia
Roger Applegate	Tennessee Wildlife Resources Agency
Robert Boyd	Pennsylvania Game Commission
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Mark W. Cunningham	Florida Fish and Wildlife Conservation Commission
Chad Dacus	Mississippi Department of Wildlife, Fisheries, and Parks
Robert W. Duncan	Virginia Department of Game and Inland Fisheries; AFWA
John R. Fischer	Southeastern Cooperative Wildlife Disease Study
Dan Forster	Georgia Department of Natural Resources
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Jonathan Sleeman	National Wildlife Health Center, USGS
Bill Stansley	New Jersey Division of Fish and Wildlife
George Timko	Maryland Department of Natural Resources
Bob Ziehmer	Missouri Department of Conservation

SCWDS OBJECTIVES

- To detect causes of sickness and death 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 FY 2013

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 agencies that support SCWDS, services will be provided to state and federal animal health authorities, public health officials, wildlife researchers, and other organizations 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 among wildlife managers and to help wildlife agencies communicate with the public and the news media.
- Provide disease evaluation services on translocated wildlife in order to assess potentials for introduction and dissemination of diseases and parasites to existing populations of wildlife, domestic livestock, poultry, or humans.
- Monitor major selected wildlife diseases to determine their impact on the 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 and federal 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

John R. Fischer, DVM, PhD, SCWDS Director and Professor
Justin D. Brown, DVM, PhD, Assistant Research Scientist
Joseph L. Corn, PhD, Senior Public Service Associate
Sonia Hernandez, DVM, PhD, Assistant Professor
Daniel G. Mead, MPH, PhD, Associate Professor
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Michael J. Yabsley, PhD, Associate Professor

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John C. Wlodkowski, BS, Research Professional I

SIGNIFICANT DEVELOPMENTS OF THE YEAR

Wildlife Mortality Investigations

The SCWDS Diagnostic Service received 653 accessions from July 1, 2012, through June 30, 2013, including 850 individual carcasses or samples. These included 328 specimens from 61 species of birds, 475 samples from 32 mammalian species, and 47 samples from 15 species of reptiles.

Species Represented Among SCWDS Diagnostic Accessions
July 1, 2012 - June 30, 2013

Birds	
American Coot	3
American Crow	4
American Goldfinch	7
American Redstart	1
American Robin	6
Bald Eagle	43
Barred Owl	2
Black Vulture	2
Black-and-White Warbler	1
Black-throated Blue Warbler	2
Black-throated Green Warbler	1
Brown-headed Cowbird	1
Canada Goose	1
Cedar Waxwing	5
Clapper Rail	3
Common Eider	9
Common Grackle	5
Common Loon	7
Common Yellowthroat	2
Cooper's Hawk	7
Domestic Chicken	1
Domestic Quail	10
Double-crested Cormorant	1
Dovekie	10
Eastern Meadowlark	3
Golden Eagle	5
Gray Catbird	1
Great Blue Heron	1
Greater Prairie Chicken	2
Great-horned Owl	4
Hispaniolan Parrot	2
Horned Grebe	4
House Finch	5
House Sparrow	1
Least Tern	2
Lesser Scaup	1
Mallard	11
Mourning Dove	3
Northern Cardinal	1
Northern Gannet	2
Northern Parula Warbler	1
Northern Pintail	6
Osprey	1
Ovenbird	2
Peregrine Falcon	4
Pine Siskin	3
Puerto Rican Parrot	20
Purple Martin	13
Red-shouldered Hawk	3
Red-tailed Hawk	10
Red-throated Loon	1
Red-winged Blackbird	14
Ross' Goose	3
Savannah Sparrow	1
Screech Owl	1
Sharp-shinned Hawk	1
Swallow-tailed Kite	1
Swamp Sparrow	3
White-throated Sparrow	1
Wild Turkey	56
Yellow-rumped Warbler	1
Total	328
Mammal	
American Black Bear	5
Big Brown Bat	6
Bobcat	2
Brazilian Free-tailed Bat	3
Coyote	2
Domestic Goat	1
Eastern Cottontail Rabbit	6
Eastern Gray Squirrel	7
Eastern Red Bat	3
Elk	39
Evening Bat	1
Fallow Deer	9
Feral Swine	1
Fisher	2
Florida Panther	2
Fox Squirrel	4
Gray Fox	7
Little Brown Bat	3
Mexican Free-tailed Bat	4
Mule Deer	5
Muntjac deer	2
Muskrat	1
Pocket Gopher	1
Raccoon	9
Red Deer	1
Red Fox	1
River Otter	1
Sika Deer	2
Striped Skunk	10
Virginia Big-eared Bat	1
Virginia Opossum	4
White-tailed Deer	330
Total	475
Reptiles	
American Alligator	7
Barbour's Map Turtle	1
Black Racer	5
Common Slider	1
Copperhead	2
Eastern Box Turtle	3
Eastern Kingsnake	1
Eastern River Cooter	2
Gopher Tortoise	2
Gray Ratsnake	2
Milk Snake	1
Northern Water Snake	1
Queen Snake	2
Rough Green Snake	1
Timber Rattlesnake	16
Total	47
Cumulative	850

Interesting or previously unreported diagnoses included: right kidney aplasia in a Puerto Rican parrot; *Salmonella* sp. infection in the brain of a brown-headed cowbird from Georgia; pansteatitis in a great-blue heron from Maryland; microcystin exposure and possible intoxication in mallards from Maryland; *Yersinia pseudotuberculosis* infections in two eastern cottontail rabbits from West Virginia; tularemia in eastern cottontail rabbits from Arkansas, Kentucky, and Maryland and an eastern gray squirrel from Kentucky; meningeal worm infestations in elk from Kentucky; pseudorabies in a Florida panther; ethylene glycol toxicosis in a raccoon from North Carolina; and a compound odontoma in a white-tailed deer from North Carolina.

Hemorrhagic Disease

Most hemorrhagic disease-related work during this year was dedicated to epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) diagnostics. For the 2012 HD season, 201 orbiviruses were isolated and identified from 346 submissions; most (n=131) were EHDV-2. There were 56 cases of EHDV-6 from Arkansas, Florida, Illinois, Indiana, Iowa, Louisiana, Kentucky, Michigan, Mississippi, Missouri, and Wisconsin. Additionally, there were 8 EHDV-1 viruses isolated (Florida, Georgia, Indiana, Kansas, Michigan, and Missouri), 1 BTV-10 (Colorado), 1 BTV-11 (Florida), 4 BTV-13 (Florida, Missouri, and bighorn sheep from Colorado).

Diagnostic work related to hemorrhagic disease during the winter and spring of 2013 has been limited to 10 cases from Florida, Georgia, Louisiana, Mississippi, Montana, North Carolina, Pennsylvania, and Wisconsin; 8 have tested negative for EHDV and BTV as of July 11, 2013, and 2 are currently being processed. Samples from Pennsylvania and Wisconsin were from previous year (2012) and had been frozen until they were submitted for testing this past spring.

Hemorrhagic Disease Research

Update on Epizootic Hemorrhagic Disease 6 - Is it genetically similar to previous years or are new recombinants responsible for 2012 outbreaks?

Epizootic hemorrhagic disease virus (EHDV) is an arthropod-borne virus. The genome is composed of ten double stranded RNA segments. Historically, only EHDV-1 and EHDV-2 were endemic in the United States. However, in 2006 exotic strains, EHDV-6, were isolated from white-tailed deer in Indiana and Illinois. In 2007, this virus was isolated from deer in Missouri; in Texas and Kansas in 2008; in Michigan in 2009; and in Arkansas in 2010. In 2012, the virus was isolated from deer in Florida, Kentucky, Louisiana, Mississippi, and Wisconsin. Despite heavy surveillance, no EHDV-6 viruses were isolated in 2011. From 2005 through 2010, one to six viruses were isolated per year, however; during 2012, more than 50 isolates were obtained from 12 states.

The exotic EHDV-6 virus is a recombinant virus, with VP2 and VP5 (the outer capsid proteins) being derived from the (EHDV-6) CSIRO 753 strain and the VP7 derived from the EHDV-2 (Alberta strain). The EHDV-6 isolates from samples collected before 2012 were separated into clusters based on analysis of partial VP7 gene. Spatial and temporal associations indicate that continual reassortment of VP7 between EHDV-6 and EHDV-2 potentially was occurring. Due to the record number of isolates and apparent geographical expansion of EHDV-6 in 2012, we sequenced a partial gene fragment of VP7 of the EHDV-6 isolates from 12 different states to assess the viral expansion seen in 2012 and identify any additional reassortment events within VP7.

No novel reassortment events were identified in VP7 in the EHDV-6 isolates from 2012. Those isolates are closely genetically related but are widely distributed in space and time. The isolates from Arkansas, Florida, Illinois, Indiana, Kentucky, Louisiana, Mississippi, and Missouri

all form a monophyletic clade ancestrally related to the EHDV-6 isolate from Texas in 2008; whereas, the EHDV-6, from Maryland is more closely related to the EHDV-6 isolates from Kansas in 2008 based on the partial VP7 sequence. Based on these data we can conclude the EHDV-6 isolates that were identified in 2012 were not novel reassortants, and they are genetically very closely related to the viruses that were circulating in previous years. Full-length genome sequencing of 12 EHDV-6 isolates is underway to asses more of the genome than the VP7.

Fatal Hemorrhagic Disease Virus (Serotype 2) Infection in an Alpaca (Vicugna pacos) from Pennsylvania

In late September of 2012, a female alpaca from Adams County, Pennsylvania, was found dead one day after displaying lethargy, anorexia, and oral ulcerations. Epizootic hemorrhagic disease virus (EHDV) serotype 2 was isolated from spleen and lung samples from this animal that were submitted to SCWDS for orbiviral isolation.

The EHD type-2 virus most often is associated with hemorrhagic disease in white-tailed deer (WTD) (*Odocoileus virginianus*). Although alpacas and other camelids historically were thought to be resistant to EHDV, bluetongue virus (BTV) infections in camelids have been reported on two occasions: In 2007, a fatal BTV (serotype 1) infection was described in an alpaca in Europe, and in 2010, BTV-related mortality was reported in an alpaca in California.

Genetic analysis of the EHDV-2 VP2 gene I, which encodes an outer glycoprotein involved in cell attachment, indicated that the alpaca EHDV-2 was 99.6% similar to an isolate from a WTD that died in Cumberland County, Pennsylvania, in the 2012 hemorrhagic disease outbreak. This suggests that both viruses originated from a common source and that these viruses can be shared between numerous hosts, including camelids, during outbreaks. To our knowledge, this is the first report of EHDV mortality in a camelid in North America, and it broadens the list of domesticated animal species that were affected by EHDV-2 in 2012.

Culicoides Surveillance

In view of the isolation of exotic EHD and BT viruses from deer, as well as detection of several exotic orbiviruses among livestock in Florida in recent years, SCWDS, following consultation with USDA-APHIS-VS, has conducted a surveillance program for endemic and exotic species of *Culicoides* spp. midges in the southeastern United States.

Surveys for *Culicoides* spp. began in November 2007 and are ongoing. Collections are made with sets of 8-10 CDC light traps per site per night. During the July 2012-June 2013 period, insect collections were conducted at 31 sites in 6 states throughout the southeastern United States and in Michigan for a total of 622 trap nights (see table below). One site in Mississippi had previous instances of exotic BT virus isolation, and the Michigan surveys were conducted in an area of an active HD outbreak prior to insect trapping. Most surveys throughout the Southeast were conducted from August through September to coincide with maximum biting midge activity and BTV/EHDV transmission.

Planning for surveys to be conducted in 2013 is underway. Surveys will be concentrated in Alabama, Arkansas, Georgia, Louisiana, Mississippi, South Carolina, and the panhandle of Florida during August-September 2013.

July 2012-June 2013

	Field Work			Lab Work			
	Total Sites	Total Counties	Trap Nights	Traps Sorted	Total *Culicoides*	Slides	IDs
Florida	4	4	117	124	14	63	233
Georgia	3	3	89	89	2816	31	180
Alabama	5	10	110	110	1681	58	212
Mississippi	9	11	180	177	6763	10	93
Missouri	0	0	0	0	0	0	0
Louisiana	6	12	110	109	7604	50	292
Arkansas	0	0	0	0	0	0	0
Texas	0	0	0	0	0	0	0
Pennsylvania	0	0	0	0	0	0	0
Michigan	4	4	16	16	94	5	89
All States	31	44	622	625	18,972	217	1,099

From July 2012 to June 2013, a total of 18,972 *Culicoides* midges were sorted and counted. Trap contents are processed at the SCWDS office in Athens, Georgia. Individual midges are identified, with a selection dissected and slide-mounted. During this period, 217 *Culicoides* spp. specimens were mounted, and 1,099 individual specimens were identified to species. Specimens processed from July 2012 to June 2013 included representatives of 31 different species of the genus *Culicoides*. However, since the project's inception, 51 total species have been identified. Processing and identifying of specimens is ongoing.

Florida: Insect trapping continued year-round in Florida from the project's inception in November 2007 through December 2011. During July 2012 to June 2013, four sites were surveyed for *Culicoides* in the Florida panhandle. Since trapping began in 2007, 31 species of *Culicoides* spp. biting midges have been identified from Florida sites. The most common species collected is *Culicoides insignis*, which was identified from 31% of Florida traps, and present at 78% of Florida sites sampled. The second and third most common species are *C. edeni* (present in 7% of traps, 41% of sites) and *C. stellifer* (present in 6% of traps, 31% of sites). Several species collected from peninsular Florida are known as tropical/subtropical species with distributions mostly in areas of Central and South America and/or the Caribbean. Their distributions in Florida are at the expected northernmost extent of their range; however, we have collected at least two of these species outside of this range in other southeastern states. The species found at various sites around Florida, include *C. barbosai*, *C. edeni*, *C. floridensis, C. knowltoni*, *C. pusillus*, and *C. insignis*. We also have identified *C. insignis* from one site in southern Georgia, one site in central Alabama, three sites in southeastern Mississippi, and one site in north-central Mississippi, as well as *C. barbosai* from single sites in southwestern Georgia and south-central Louisiana. See the next section on *Culicoides* distribution for more information on species we have found in Florida and outside their expected ranges.

Culicoides sonorensis, a species implicated in BTV transmission, has only been identified from two sites in Florida (Longino Ranch, Sarasota County; Ewenity Farm, Manatee County). At each site *C. sonorensis* was found in only one trap (2% of traps at Longino Ranch; 0.3% of traps at Ewenity Farm). Both sites had cattle and/or sheep present and are located on the central Gulf Coast. Ewenity Farm was also positive for exotic BTV in past years. At both sites *C. insignis* was the most common species trapped (present in 80% of traps at Longino Ranch; 65% of traps at Ewenity Farm).

Georgia: During July 2012 to June 2013, three sites were surveyed for *Culicoides* in Georgia. Since trapping began in 2008, 23 of *Culicoides* spp. biting midges have been identified from Georgia sites. The most common species collected were *C. haematopotus* (in 36% of GA traps, 86% of GA sites); *C. stellifer* (in 26% of GA traps, 93% of GA sites); and *C. debilipalpis* (in 10% of GA traps, 64% of GA sites). Of note, one specimen of *C. insignis* was trapped in Dooly County in southwestern Georgia in August 2012. Additionally, another specimen of *C. insignis* was trapped in Long County, Georgia (southeastern Georgia, but not coastal). This species has been implicated in BTV transmission, and while it is common in Florida, it is an unusual catch north of Florida. Also of note, two specimens of *C. alachua* were trapped in Ware County, Georgia (southeastern Georgia, but not coastal). Another possible state record, we previously identified this species in Alabama. *Culicoides alachua* belongs to the subgenus Avaritia of which at least one species (*C. obsoletus*) has been implicated in BTV transmission in other parts of the world. Historically, *C. alachua* has been found in the peninsular Highlands region of Florida and into the panhandle, as well as in South Carolina. And finally, one specimen of *C. barbosai* was trapped in Dooly County in August 2012. This species is usually associated with mangrove swamps and coral sand beaches, so it is a very unusual finding for interior Georgia. *C. barbosai* is also a subtropical species found previously in Florida, throughout the Caribbean, Central America, and northern South America. See the next section on *Culicoides* distribution for more information on species we found in Georgia and outside their expected ranges.

Alabama: During July 2012 to June 2013, five sites were surveyed for *Culicoides* in Alabama. Since trapping began in 2008, 27 species of *Culicoides* biting midges have been identified. The most common species collected were *C. stellifer* (in 31% of AL traps, 100% of AL sites); *C. haematopotus* (in 30% of AL traps, 100% of AL sites); and *C. debilipalpis* (in 19% of AL traps, 89% of AL sites). Of note, several female specimens of *C. alachua* were identified from insects collected at Lowndes County WMA near Montgomery, in July 2008. In addition, a male specimen of *C. alachua* was later identified from trapping conducted at the same site in August 2008. Most recently, another female specimen was collected in September 2011 at the same site. Historically, this species has been found in the peninsular Highlands region of Florida and the Florida panhandle, as well as in South Carolina. This possible new state record may represent a range expansion for this species. Several survey sites were added in Alabama in 2009 to increase surveillance for this and other *Culicoides* species. Also of note, one female specimen of *C. insignis* was identified from insects collected in Elmore County in central AL in September 2011. We have found this species commonly throughout central and southern Florida. Several specimens have also been found throughout Mississippi, as well as one in Georgia. While not a state record, it is a rare specimen in Alabama, and is a species implicated in BTV transmission in North America. One further notable finding is two specimens of *C. neopulicaris* collected from different sites in central-western and southwestern Alabama. These specimens represent a possible new state record for Alabama. We have previously found this species only in southern Texas. It is a tropical/subtropical species with a published range from Louisiana and Texas south to Costa Rica. It belongs to the subgenus *Culicoides* and the *pulicaris* complex, from which *C. pulicaris*, a species implicated in BTV transmission in Europe, also belongs. See the next section on *Culicoides* distribution for more information on species we found in Alabama and outside their expected ranges.

Mississippi: During July 2012 to June 2013, nine sites were surveyed for *Culicoides* in Mississippi. One site is a location where exotic BTV has been identified (private land in Yalobusha County). Since trapping began in 2008, 26 species of *Culicoides* have been identified from Mississippi sites. The most common species collected were *C. haematopotus* (in 53% of MS traps, 100% of MS sites); *C. stellifer* (30% of MS traps, 85% of MS sites); *C. debilipalpis* (in 22% of MS traps, 90% of MS sites); and *C. arboricola* (in 12% of MS traps, 90% of MS sites). Of note, one specimen of *C. kirbyi* was identified from south-central Mississippi and may be a new state record, as it has only been reported from Alabama and Maryland. Also

of note, nine specimens of *C. insignis* have been identified from four different sites in Mississippi. Three sites are located in southeastern Mississippi, and one site is in the north-central part of the state. We have now identified this species in collections made in 2008, 2009, and 2010. This collection may represent a new state record for this species (which is also implicated in BTV transmission). See the next section on *Culicoides* distribution for more information on species we found in Mississippi and outside their expected ranges.

Louisiana: During July 2012 to June 2013, six sites were surveyed for *Culicoides* in Louisiana. Since trapping began in 2008, 18 species of *Culicoides* biting midges have been identified from Louisiana sites. The most common species collected were *C. haematopotus* (in 65% of LA traps, 100% of LA sites); *C. arboricola* (in 21% of LA traps, 86% of LA sites); and *C. stellifer* (in 21% of LA traps, 79% of LA sites). *C. debilipalpis*, which is not as common but a widespread species, was only present in 17% of all Louisiana traps but has been found at 93% of Louisiana sites. Of note, one male specimen of *C. barbosai* was identified from a site in south-central Louisiana, west of Baton Rouge (trapped in August 2012). We have also found this subtropical midge species in interior Georgia, as well as throughout south Florida. Because it is usually associated with mangrove swamps and coral sand beaches, it is a very unusual finding for interior Louisiana. We plan to return to this site during the upcoming *Culicoides* trapping season to attempt to confirm this identification. See the next section on *Culicoides* distribution for more information on species we found in Louisiana and outside their expected ranges.

Michigan: During July 2012 to June 2013, assistance with *Culicoides* identification was provided to USDA-ARS Arthropod-Borne Animal Diseases Research Unit and the Michigan Department of Natural Resources. Four sites were surveyed for *Culicoides* in Michigan during an active EHD outbreak. Only four species of *Culicoides* were identified from the Michigan traps: *C. crepuscularis* and *C. venustus* (both in 25% of MI traps, 75% of MI sites), *C. haematopotus* (in 19% of MI traps, 75% of MI sites), and *C. stellifer* (in 13% of MI traps, 50% of MI sites).

Culicoides Distribution Evaluation

Data on the distribution of *Culicoides* in the Coastal Plain region of the southeastern United States accumulated in these surveys since November 2007 are being summarized, and data on species found in locations outside of previously established ranges are being prepared for publication (mostly limited to new state records or new county records). To date, 11 species of *Culicoides* have been identified from states or areas for the first time (see Table below).

***Culicoides* spp.**	**Newly recorded from:**
C. beckae	Mississippi
C. oklahomensis	Alabama, Arkansas, Georgia
C. alachua	Alabama, Georgia
C. hollensis	SW Florida (prev. only known from NE Florida, northward)
C. neopulicaris	Alabama
C. butleri	Texas
C. insignis	Mississippi
C. sonorensis	Alabama, Florida
C. barbosai	Georgia, Louisiana
C. loisae	Alabama
C. kirbyi	Mississippi

Culicoides Association with Bluetongue and Epizootic Hemorrhagic Disease

Data collected on *Culicoides* since 2007 are being analyzed in collaboration with the UGA School of Ecology to determine species distribution and emergence patterns in relation to spatial and temporal patterns of occurrences of bluetongue and epizootic hemorrhagic disease. Included are analyses of which *Culicoides* species are statistically associated with premises where exotic bluetongue and epizootic hemorrhagic disease outbreaks have occurred and which *Culicoides* species are predicted to be potential vectors.

Assistance to USDA-ARS

SCWDS is providing assistance to USDA-ARS with *Culicoides* identification. In addition to identifying specimens, SCWDS also is providing technical assistance with laboratory procedures, and providing reference specimens of *Culicoides* and literature on *Culicoides* identification.

Exotic Ectoparasite Surveillance

SCWDS is conducting surveillance for exotic ticks and other livestock arthropods in the southeastern United States through a Cooperative Agreement with USDA-APHIS-Veterinary Services. The current focus of the Exotic Ectoparasite Surveillance Project within this Cooperative Agreement is to determine the geographic area of risk for transmission of equine piroplasmosis in Texas, and to determine the status of wildlife as hosts for cattle fever ticks. Specific objectives of this project are to (1) determine the host range and geographic distribution of *Amblyomma cajennense* in Texas; (2) determine other tick species present in south Texas that may serve as vectors of equine piroplasmosis; and (3) determine wildlife hosts infested by *Rhipicephalus (Boophilus) annulatus* and *Rhipicephalus (Boophilus) microplus.* All ticks and other arthropods collected are submitted to the National Veterinary Services Laboratories (NVSL), APHIS, USDA. Data on ticks and other livestock arthropods are entered into a database and prepared for distribution to USDA-APHIS-Veterinary Services in Quarterly Reports and an Annual Report specific to the Cooperative Agreement.

Ticks and other arthropods were collected from hunter-killed white-tailed deer, javelinas and coyotes at state managed hunts at Las Palomas WMA (Cameron County), Chaparral WMA (Dimmit and LaSalle Counties), and Daughtrey WMA (McMullen County), Texas, during November 2012. Ticks and other arthropods collected were submitted to USDA-APHIS-VS-NVSL, and identifications are pending.

Ticks and other arthropods were collected from 132/200 free-ranging mammals, birds, reptiles and amphibians captured and examined at six arthropod survey sites during a six-week survey period in south Texas in May-June 2012 and were processed by USDA-APHIS-VS-NVSL and the data were received by SCWDS during the current year. Three of these sites were private ranches in Starr County and three were state wildlife management areas in Cameron, Dimmit, LaSalle, Live Oak, and McMullen counties. Tick species identified so far from these collections include: *Amblyomma sp., A auricularium, A. cajennense, A. inornatum, A. maculatum, Carios sp., Dermacentor halli, D. variabilis, Ixodes tovari, I. woodi, and Ornithodoros turicata* (all ticks collected came from mammalian hosts).

Ticks and other livestock arthropods collected from 402/497 free-ranging mammals and birds captured and examined at five arthropod survey sites in south Texas in February-March 2012 were processed by USDA-APHIS-VS-NVSL, and the data were received by SCWDS during the current year. Tick species identified from these collections include: *Amblyomma sp., A. americanum, A. auricularium, A. cajennense, A. imitator, A. inornatum, A. maculatum,*

Anocentor nitens, Argas brevipes, Carios sp., Dermacentor sp., D. albipictus, D. halli, D. variabilis, Ixodes affinis**, I. conepati, I. cookei, I. scapularis, I. texanus, I. tovari, I. woodi, and Ornithodoros turicata*. (*This tick species was the only one collected from an avian host; all other species were collected from mammals. **This is the first report of *I. affinis* in Texas.)

Planning, including applications for collection permits and permits for access to state and federal lands, was continued in order to conduct further surveys in south Texas. Contacts also are being made with private landowners to request access in south Texas. Wildlife surveys involving trapping of free-ranging wildlife in south Texas planned for October-November 2012, January-February 2013, and April-May, 2013 were canceled due to delays associated with an Endangered Species Section 7 Consultation being conducted by the U.S. Fish and Wildlife Service. These surveys will resume when the Consultation has been completed. Plans currently are being made to resume surveys in October 2013.

Four manuscripts summarizing selected findings related to this Cooperative Agreement were published. These manuscripts 1) document first reports of exotic ectoparasites recovered from free-ranging exotic reptiles in Florida, published in the *Journal of Medical Entomology;* 2) document the first at-large collection of the mite *Echimyopus dasypus* in North America, published in *Systematic & Applied Acarology*; 3) document the first at-large collection of the tick *Amblyomma parvum* in the United States, published in *Systematic & Applied Acarology*; and 4) summarize the outbreak of equine piroplasmosis in Florida, published in the *Journal of the American Veterinary Medical Association*.

One additional manuscript to summarize selected findings made through or related to this Cooperative Agreement has been submitted for publication. This manuscript documents the first record of chewing lice, *Damalinia (Tricholipeurus) lipeuroides* and *D. parallela* (Phthiraptera: Trichodectidae), on white-tailed deer in the U.S. Virgin Islands, and is in review at the *Caribbean Journal of Science*.

The following text further describes some of the results from Texas and is excerpted from Mertins, J.W. and Corn, J.L. 2013. Case Report: Equine piroplasmosis and wildlife ectoparasites in the USA, especially South Texas. VMO Observer.

"These surveys are not yet complete enough to make many generalizations or form any conclusions, but we can highlight a few of our specific, interesting, or unusual observations and findings.

The local tick fauna of south Texas is unlike any other in the United States in both the number and variety of species present. For example, only in this area are there resident sympatric populations of tick species representing every one of the currently recognized tick genera known to occur in the U.S., i.e., Amblyomma, Anocentor, Argas, Carios, Dermacentor, Haemaphysalis, Ixodes, Ornithodoros, Otobius, Rhipicephalus (Boophilus), and Rhipicephalus (Rhipicephalus). And although a few of the local tick species may feed extensively and in large numbers on domestic animals, all of them feed to some degree on local wildlife, and most of them exclusively use wild animals as hosts.

At the NVSL, the local diversity of the tick fauna in south Texas quickly became apparent when we began the identification process for the numerous immature stage ticks (i.e., larvae and nymphs) dominating many of the submitted ectoparasite samples. Adults of several of the most common tick species in our samples are readily discriminated, but their pre-adult stages often are so morphologically similar that no easy means are readily available for telling them apart. In the larval stage, such confusing species complexes include Amblyomma americanum/cajennense/imitator, Amblyomma auricularium/inornatum, and Dermacentor halli/variabilis. For nymphs of these ticks, we can distinguish A. americanum from all the others, but the remaining three species pairs are still very difficult to partition into their constituent halves. All of these species complexes are sympatric in the USA only in south Texas, and

evidently, no one else previously has dealt with the problem we faced – quickly and efficiently segregating and accurately identifying large numbers of such immature ticks. Published means are available for distinguishing larvae and nymphs of the two Dermacentor species and for the nymphs of A. cajennense and A. imitator, but they are either incomplete, unreliable, or difficult to use, e.g, the Dermacentor larvae must be mounted on slides for study under a compound microscope. At the NVSL, we now have devised some provisional means for distinguishing all of these species as immatures, except for the larvae of A. cajennense/imitator.

In addition to the cited confusing species pairs in south Texas, several other local ticks can present some unusual challenges for identification, e.g., some occur in the USA only here, many occur only on wildlife, and most are not commonly encountered in routine diagnostic work. Some examples include Anocentor nitens, Ixodes conepati, I. eadsi, I. tovari, and C. dugesi. Indeed, the larval stages of the latter two species – both of which we have encountered already – are not even described in the tick literature.

Although we are concentrating on ticks, as indicated earlier, we also are opportunistically collecting and identifying other arthropods from our available hosts. Thus far in this effort, we have identified over 75 species of non-tick ectoparasites, comprising dipterans, fleas, chewing and sucking lice, and a wide variety of astigmate, mesostigmate, and prostigmate mites. Many of the individual acarine taxa we have encountered, particularly the feather mites on our bird hosts, represent novel forms not previously described in the scientific literature, further emphasizing how little is known about the ectoparasite fauna of South Texas."

Avian Influenza Surveillance

Surveillance and research continued in order to better understand the epidemiology of avian influenza viruses (AIV) and avian paramyxoviruses (APMV) within North American Charadriiformes (shorebirds and gulls) with funding support provided through the Minnesota Center of Excellence for Influenza Research and Surveillance (MCEIRS-NIH), DHS, and USDA.

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:

- Completed a study related to a co-infection experimental trial in mallards to determine the effects of APMV-1 on AIV shedding and pathogenesis.
- Completed analysis of AIV serologic and virologic results from more than 3,000 passerines sampled in Minnesota, New Jersey, and Georgia. Antibodies were detected in very few (<0.3%) birds and infections were not detected by RT-PCR or virus isolation.
- During late summer/fall migration, collected and tested cloacal swab and serum samples from more than 2500 ducks in Louisiana, Minnesota, and Texas; more than 300 influenza viruses were isolated.
- During spring migration, collected and tested swab samples from more than 1,000 blue-winged teal and green-winged teal in Louisiana and Texas; viruses were isolated from both states with H7 subtypes predominating.
- Collected and tested approximately 1,000 samples (cloacal swabs, fecal samples, serum) from shorebirds at Delaware Bay, New Jersey.
- Collected and tested over 400 samples from migrating ruddy turnstones from Georgia and Florida sites prior to Spring migration. H12 influenza viruses were isolated.
- Attached 74 geolocators to ruddy turnstones wintering in Brazil.

- In collaboration with St Jude Children's Research Center, a pathogenesis study was completed to determine if H7 viruses derived from wild birds will replicate and cause disease in a mammalian model (DBA/2j mice).
- A study was completed on the phylogenetics of recent H7 from Eastern Asia; additional work related to the recent evolutionary history of H7 influenza viruses in North America is in progress.
- In collaboration with the Alaska Science Center, USGS, and the Southeast Poultry Research Laboratory, ARS-USDA, we completed a serologic study of northern pintails in Japan testing for antibodies to influenza, West Nile, and Japanese Encephalitis viruses.

Wild Bird H7N9 LPAI Virus Surveillance

During the spring of 2009 and 2011, multiple domestic poultry flocks with antibodies to H7N9 AI virus were identified through routine pre-slaughter program testing, and a few virus isolations were made. Affected flocks were present in multiple states, including Illinois, Kentucky, Minnesota, and Tennessee in 2009, as well as Minnesota and Nebraska in 2011. Through a cooperative agreement with USDA-APHIS-VS, SCWDS provided support to the ongoing investigations to better understand the epidemiology of this virus in domestic poultry and evaluate the risks for wild bird involvement. Specifically, our objectives were: 1) to conduct wild bird inventories on all farms affected during 2009 in order to determine the potential risk for AI virus spill-over from wild birds to domestic poultry; and 2) to characterize the genetic diversity of H7 AI viruses isolated at SCWDS from North American wild birds from 2000-2010. For Objective 2, we compared the sequences from the H7N9 AI poultry viruses to our SCWDS wild bird isolates and existing H7 poultry sequences in GenBank in order to identify a potential source. Additionally, we have expanded on the proposed work and conducted active surveillance for AI virus in wild bird species on two of the farms affected in Minnesota during 2011, through virus isolation and serology. We have completed this research. A manuscript describing the genetic analysis is currently being reviewed by co-authors and will be submitted to a peer-reviewed journal upon completion. The surveillance data have been included in a larger serosurveillance study for influenza in passerines. This manuscript is currently being written and will be submitted to a peer-reviewed journal upon completion.

Avian Influenza Research

H5N1 HPAI Experimental Infection Trials

In collaboration with Dr. David Swayne of the USDA-ARS Southeast Poultry Research Laboratory (SEPRL), SCWDS personnel completed an experimental trial in bar-headed geese and ruddy shelducks to evaluate clinical disease and viral shedding associated with a subclade 2.3.2 H5N1 HPAI virus. This subclade has become the most common strain of H5N1 HPAI detected in wild birds in Eurasia, and the goal of this trial was to provide insight into the ever changing ecology of this virus. This manuscript has been accepted for publication in the journal *Veterinary Pathology*.

LPAI Experimental Infection Trials in Ducks and Gulls

In support of funding provided through Minnesota Centers of Excellence for Influenza Research and Surveillance (MCEIRS)-NIH, multiple experimental trials were performed to better understand the epidemiology of AI virus in ducks and gulls; two groups that are recognized reservoirs for AI. During this fiscal year, SCWDS conducted the following activities:

- Completed a study to characterize the concentration and duration of fecal excretion of LPAI virus by mallards, the dominant route of shedding by one of the most important reservoir hosts. Diagnostically important questions were addressed in this study by comparing RT-PCR (Ct values) to viral titrations and cloacal swabs to fecal samples. A manuscript describing this research was published in the *Journal of Wildlife Diseases*.
- Completed a study to evaluate the susceptibility of ducks, chickens, turkeys, and gulls to H13 LPAI viruses. This subtype has long been thought to be highly gull-adapted, but little else is known about the epidemiology and/or pathobiology of these influenza viruses. A manuscript describing this research was published in *Avian Diseases*.
- Completed an experimental trial in mallards to better define the effects that route of exposure have on duration and extent of LPAI virus shedding in mallards. A manuscript describing this research was published in *Avian Diseases*.
- Completed a study to evaluate the susceptibility of mallards to wild-type, non-egg passaged LPAI viruses. The goal of this study was to provide insight into what our egg-based diagnostic results actually mean in regards to infectivity in wild bird hosts (mallards). A manuscript describing this research was published in the *Journal of Wildlife Diseases*.
- Completed a study to evaluate the pathogenesis of LPAI virus infection in mallards. Specifically, we characterized the microscopic lesions and distribution of viral antigen over the course of LPAI virus infection in mallards. A manuscript describing this research was published in *Avian Diseases*.
- Completed a study to evaluate the susceptibility of European starlings and house sparrows to swine influenza viruses. The objective of this study was to evaluate the risk for these peridomestic species to be infected with swine influenza viruses. A manuscript describing this research was published in the *Journal of Wildlife Diseases*.
- Completed a study to evaluate the effects that genetic reassortments have on the biology of mallard-origin LPAI viruses, specifically persistence in water and infectivity for the mallard host. A manuscript describing this research was published in the *Proceedings of Royal Society B*.
- Completed a study to evaluate interactions between avian influenza virus and avian paramyxovirus co-infections in mallards. A manuscript describing this research has been prepared and is currently in review.

Collectively, these studies have provided valuable data that improved our understanding of LPAI virus infection and transmission in important wild bird reservoirs. Additionally, the data from these experimental trials can be used to support ongoing field studies, both for interpreting surveillance results and also highlighting ways to improve future sampling efforts.

Lymphoproliferative Disease Virus Surveillance and Research

During 2009, three cases of lymphoid neoplasia were identified in adult wild turkeys submitted to the diagnostic service at SCWDS. Multiple tissues from all three turkeys tested positive for lymphoproliferative disease virus (LPDV), a poorly understood avian retrovirus that previously had been associated with disease in domestic turkeys in Europe and Israel, and was thought to be exotic to North America. Since the 2009 cases, SCWDS has screened wild turkey diagnostic submissions and identified additional positive turkeys throughout the eastern United States. A manuscript describing the genetic characterization of these North American wild turkey LPDV isolates is currently being reviewed by co-authors and will be submitted for publication in a peer-reviewed journal. Additionally, we have tested tissues from apparently asymptomatic hunter-killed wild turkeys from multiple states throughout the eastern United States for LPDV. The objective of this study is to better understand the epidemiology of LPDV

and provide baseline information on the prevalence of LPDV infection in wild turkeys. Samples have been collected for this project, and testing is underway.

Canada Goose Serology Study

During the past several years, we have been evaluating Canada geese as sentinels for detecting areas where avian influenza virus (AIV) transmission is occurring by comparing the prevalence of AIV antibodies in Canada geese to prevalence of AIV isolated during previous studies on dabbling ducks.

Samples were collected from 3,782 geese in five states (Minnesota, New Jersey, Pennsylvania, Washington, and Wisconsin) from 2009-2012, and 44 Canada goose eggs in 2012. Overall, 930 (25%) samples had antibodies to AIV. A subset (*n*=200) of these positive samples was tested for subtype-specific antibodies to six hemagglutinin subtypes: All of the subset tested had antibodies against the H5 subtype, 52% were positive for H3, 26% were positive for antibodies to H4, 41% were positive for antibodies to H6 viruses. The remaining two suptypes H7 and H9 that were tested had antibody prevalences at 7% and 9% respectively. In addition, 66% (29/44) of Canada goose eggs had antibodies to influenza A viruses detected in their yolk-sac. This research indicates that Canada geese are exposed to the main subtypes known to be most commonly isolated from dabbling ducks (H3, H4, and H6); however, they are also frequently exposed to viruses of the H5 subtype, which is not known to circulate at high prevalences in ducks or other influenza A virus reservoirs in North America. Additionally, we were able to detect antibodies in yolk-sac, indicating that passive transmission of antibodies from mother to goslings is likely; however, further work is needed to completely understand if this occurs and how long maternal antibodies persists.

Avian Influenza Diagnostics Research

As part of a collaborative study with the USDA-ARS-SEPRL, we completed two studies to evaluate the performance of commercially-available serologic assays, including type- and subtype-specific (H5) assays. All commercial kits were in bELISA formats. The manuscript on type-specific serologic testing was published in the *Journal of Veterinary Diagnostic Investigations* and the study on subtype-specific serologic testing was accepted for publication in *Influenza and Other Respiratory Viruses*. Another study was completed that evaluated the use of FTA® sampling cards for molecular detection of avian influenza virus in wild birds. A manuscript describing these results was published in *Avian Diseases*. The serology research detailed above was funded by the MCEIRS-NIH grant.

Wellfleet Bay Virus in Common Eiders

Prevalence and Geographic Distribution

A collaborative project was initiated involving SCWDS, USGS National Wildlife Health Center, U.S. Fish and Wildlife Service, USDA-APHIS Wildlife Services, Tufts University, Maine Department of Inland Fisheries and Wildlife, Environment Canada, University of Montreal, and University of Quebec at Montreal to assess the prevalence and geographic distribution of Wellfleet Bay virus (WFBV) exposure in *(Somateria mollissima)* in the eastern United States and Canada. WFBV is a recently identified Orthomyxovirus associated with annual common eider mortality events near Cape Cod, Massachusetts. Common eiders are large, pelagic ducks of considerable ecologic and economic importance.

Nothing is known about the transmission mechanisms, host-range, or epidemiology of this virus, or its potential impacts on common eider populations. This study will use a

microneutralization assay to look for antibodies against WFBV in serum samples from common eiders. The prevalence of antibodies will be compared by location, season of collection, and demographic characteristics to look for patterns of exposure. The goal is to use this information to direct future research into the epidemiology of this virus. The majority of these eider serum samples also will be screened for antibodies to influenza A viruses as part of a larger collaborative study evaluating the role of sea ducks in the ecology of avian influenza virus.

We have received approximately 2,432 serum samples collected from 2007 through 2013. These samples have been cataloged, and serologic testing is underway.

Experimental Infection Trial

A collaborative project was continued between SCWDS, USGS National Wildlife Health Center, and U.S. Fish and Wildlife Service to conduct an experimental infection trial in common eiders with Wellfleet Bay virus (WFBV). This study was conducted at the USGS National Wildlife Health Center in Madison, Wisconsin, in the summer of 2012. WFBV is a recently identified orthomyxovirus associated with annual mortality events in common eiders. Preliminary genetic analysis indicates that this virus may be related to a group of arthropod-borne, avian orthomyxoviruses, but a vector has not been identified. Determining viable routes of WFBV transmission is a significant research priority this study will address.

This study was designed to compare the effects of different exposure routes on the infection and development of clinical disease in common eiders. It also examined patterns of viral shedding, rates of seroconversion, and the pathogenesis of viral infection. Laboratory testing is currently underway. Samples collected during the trial have been used to compare and validate diagnostic tests for future WFBV surveillance efforts.

Chronic Wasting Disease Surveillance

Surveillance for chronic wasting disease (CWD) of cervids in the Southeast has continued. Samples from 5,078 white-tailed deer and 5 elk were submitted to SCWDS for CWD testing by enzyme-linked immunosorbent assay (ELISA) testing as part of the active CWD surveillance programs for Florida, Louisiana, Mississippi, Missouri, Tennessee, and West Virginia. SCWDS received samples from 71 white-tailed deer and 9 elk submitted as clinical cases through the diagnostic service, and 36 white-tailed deer submitted from herd health examinations conducted in three states (Georgia, North Carolina, and West Virginia) for CWD testing by ELISA. Samples from four red deer submitted as part of Tennessee's active CWD surveillance were sent to the National Veterinary Services Laboratory (NVSL) at Ames, Iowa, for CWD testing by immunohistochemistry (IHC), because the ELISA test used by SCWDS is not labeled for red deer. Additionally, formalin-fixed samples from 13 white-tailed deer, 4 elk, and 1 red deer that were received as clinical cases through the diagnostic service were sent to NVSL for CWD testing by IHC. As of June 30, 2013, testing has been completed on all but 38 of the ELISA samples. Evidence of CWD was detected in 21 samples, including 16 white-tailed deer from West Virginia and five white-tailed deer from Missouri, all tested through the active surveillance programs for each state. No evidence of CWD was detected in any of the remaining samples tested, including those submitted through the diagnostic service.

Since October 2002, the SCWDS laboratory has tested samples from 107,028 cervids using IHC or ELISA, and has detected CWD in 146 animals. These samples include active surveillance and passive surveillance specimens. SCWDS has been testing lymph node and brainstem samples from all target profile animals submitted since 1997. Target animals are adult deer or elk that are emaciated and showing some combination of clinical signs, including abnormal behavior, increased salivation, tremors, stumbling, incoordination, difficulty

swallowing, excessive thirst, and excessive urination. To date 1,874 target profile animals from CWD surveillance programs, and 26 target animals from 1971-1997 for which brain or lymph node samples were available, have been tested. CWD was not detected in any of the 87 target animals tested this past year. There have been five target profile animals in total that have tested positive for CWD, all from the active, state CWD surveillance programs.

CWD Program Development

SCWDS continued 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. In addition to extensive telephone and electronic communications, SCWDS personnel traveled to Lakeland, Florida, to provide a presentation on chronic wasting disease to the Florida Fish and Wildlife Conservation Commission that is considering a ban on the importation of live cervids into the state.

SCWDS also assisted state wildlife management agencies with proposed regulations designed to reduce the risk of CWD introduction via importation of live cervids, with development of CWD surveillance strategies, and kept the states advised regarding the Federal Interim Final Rule on CWD Certification and Interstate Movement for Captive Deer, Elk, and Moose that was published in 2012. Dr. John Fischer chairs the Association of Fish and Wildlife Agencies' CWD Working Group and served as a member of the Working Group established by APHIS-Veterinary Services to revise the CWD Program Standards document that also was published in the summer of 2012. This Working Group met via weekly conference calls from November-May.

Artificial Management Activities and Disease Risks

SCWDS assisted states during the past year as they dealt with policy and/or legislative issues that could impact wildlife health. The issues fall into the general category of highly artificial wildlife management activities including private ownership and propagation of wildlife, high fence shooting enclosures, baiting and feeding, etc.

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 over 37,000 humans in the United States. During this period, over 1,549 human deaths were attributed to WNV infection. During 2011, 5,674 human cases, including 268 fatal cases, were reported from 43 states and the District of Columbia. New cases occurred from January to December.

From 1999 to 2012, WNV was detected in over 64,000 dead birds representing more than 308 species. During nationwide surveillance in 2012, WNV was detected in 2,470 dead birds, 690 horses, and 22,778 mosquito pools. The virus, or viral RNA, has been detected in at least 60 species of mosquitoes in the United States since 1999.

During the last fiscal year, SCWDS continued to conduct West Nile virus surveillance among wild birds and mosquitoes in Georgia through contracts with Chatham County Mosquito Control, DeKalb County Board of Health, and Clarke Mosquito Control.

During the last fiscal year, 6,014 mosquito pools and tissue samples from 10 dead birds submitted by Georgia county health departments were evaluated for WNV infection. West Nile virus was detected in 113 mosquito pools. Additional viruses detected in mosquito pools were Flanders virus (33 pools), a genetic variant of Flanders virus (2 pools), and eastern equine

encephalitis virus (3 pools). A single mosquito pool was found to be co-infected with Flanders virus and West Nile virus. West Nile virus was not isolated from dead wild bird samples.

West Nile Virus Genetic Diversity

In response to the emergence of WNV in the United States in 1999, SCWDS has collaborated with the Georgia Department of Public Health to provide laboratory support for the State's WNV surveillance efforts. Since 2001, over 3,000 dead wild birds and 40,000 mosquito pools have been tested for WNV. To date, SCWDS has isolated the virus from over 2,500 samples that originated throughout Georgia. Our primary objectives were to determine the temporal and spatial distribution of WNV in Georgia, identify important WNV vectors, provide guidance to local mosquito control programs, and to assist local health departments in assessing human risk and preventing human infections.

We sequenced the premembrane (preM) and envelope (E) genes (2004bp) from 111 isolates collected from 2001 to 2011 from all over Georgia. Additionally, in order to assess viral gene flow from other geographical regions in the United States we combined our data with previously sequenced isolates from other states. Phylogenetic analysis indicates that WNV entered Georgia through multiple introductions over the course of the past decade. We first detected WNV in a dead crow (*Corvus brachyrhynchos)* that was sent to SCWDS on July 10, 2001, from Lowndes County (south Georgia). Additionally, WNV positive samples from a crow and a Cooper's hawk (*Accipiter cooperii)* were received within two weeks of the first positive detection in Georgia. Based on the surveillance data, it is unclear if WNV was introduced via a single introduction into the state and then subsequently spread, or if it was due to multiple introductions. Our results from the preM and E genes suggest that the rapid spread of the virus in the state of Georgia in 2001 may have originated from viruses that were circulating in Florida and the northern United States. However, it is possible that an intermediate transition between progenitor and isolate occurred. Full-length genome sequencing is underway to resolve this matter further.

Several interesting trends have been noted since surveillance was initiated in 2001. In Savannah, Georgia, WNV was detected from 2003-2007, but not from 2008-2010 (despite intense surveillance). In 2011, Savannah experienced a large epidemic and WNV was detected in over 300 mosquito pools. Based on the phylogenetic data, it appears that WNV was reintroduced into Chatham County by a long distance migration event from the northeastern United States. The isolates from Chatham County in 2011 have two amino acid differences when compared to the NY99 strain. These mutations occurred at position A29S in the preM and in the E gene, K406E. Additional surveillance in Chatham County from 2012 will provide information as to whether this dominant strain has become fixed despite continued mosquito control.

Our results collectively indicate that the effectiveness of local vector control efforts may be limited by viral import and recolonization. In Georgia, WNV is continually reintroduced from large and small-scale migration events, thus indicating that continued surveillance is essential as this virus continues to evolve.

Foreign Animal Disease Preparedness, Surveillance, and Response

SCWDS maintains a roster of State Wildlife Liaison Officers for Emergency Programs, USDA-APHIS. These persons are appointed by their respective state wildlife agency to assist the USDA during animal disease emergencies. All SWLOs are contacted every two years to update their availability status and contact information. The most recent update was completed

in 2013. SWLOs who retire or become unavailable are replaced as needed, and several replacements were made during the current year.

Wildlife Seminar for Emergency Animal Disease Preparedness

SCWDS conducted the Wildlife Seminar for Emergency Animal Disease Preparedness in Athens on May 14-16, 2013, at The University of Georgia's Center for Continuing Education. Participants included 14 veterinarians representing USDA-APHIS-Veterinary Services, 8 wildlife biologists/State Wildlife Liaison Officers representing state wildlife management agencies, and 3 veterinarians representing state agriculture agencies. The training course is provided as part of the Cooperative Agreements with USDA-APHIS-Veterinary Services and Wildlife Services and has been well received by the attendees. To date, SCWDS has provided training for over 500 USDA and other regulatory agency veterinarians to heighten the level of awareness regarding wildlife aspects of foreign animal diseases and emergency response.

The objectives of the Seminar were to:

- Familiarize Foreign Animal Disease Diagnosticians with wildlife management in the United States and the relationship that this profession has with our livestock and poultry economy.
- Familiarize USDA Emergency Response State Wildlife Liaison with the threats of foreign animal diseases and emergency response.
- Delineate jurisdiction of authority and areas of responsibility for this nation's wildlife resources and define those agencies and organizations involved.
- Improve liaison between Veterinary Services, APHIS, USDA, and State and Federal Fish and Wildlife Agencies throughout this country.
- Review some salient diseases of wildlife with consideration for the potential involvement of domestic livestock and poultry.
- Appraise possible involvement of wildlife in the event of a foreign animal disease introduction, with emphasis on the impact that this would have upon eradication measures.
- Afford opportunities for discussing problems of mutual concern for those people working with diseases of domestic livestock and poultry and wildlife.

The first two days of the seminar followed a presentation and discussion format, while day three primarily was devoted to emergency response to animal disease, including a test exercise. Course instructors included individuals from USDA-APHIS-Veterinary Services, USDA-APHIS-Wildlife Services-National Wildlife Disease Program, Centers for Disease Control and Prevention**,** University of Tennessee**,** University of California at Davis, Oregon Department of Fish and Wildlife, Michigan Department of Natural Resources**,** Minnesota Department of Natural Resources, Nevada Department of Wildlife, The University of Georgia, and SCWDS. The seminar received highly complementary evaluations from participants and speakers.

Natural History of Piroplasms and Other Tick-Borne Pathogens

The project to characterize piroplasms and other tick-borne pathogens of wildlife was continued. In an effort to better understand piroplasms transmitted by ticks, testing of questing or human-fed ticks from several southeastern states was continued. A piroplasm-specific PCR was used to screen 1,632 ixodid ticks from Georgia (n=486), Kentucky (n=103), Tennessee (n=626) and Texas (n=416) that were questing (n=42), or collected from animals (n=627), and humans (n=963). The study focused on *Dermacentor variabilis* (n=702) and *Amblyomma americanum* (n=743), but other ticks were tested, including *A. maculatum* (n=16), *A. cajennense* (n=89), *Ixodes scapularis* (n=4), *I. woodi* (n=1) and unspeciated *Amblyomma* (n=77). Few ticks

were infected, as 37 (2.3%), 36 (2.2%), and 9 (0.6%) were positive for *Theileria*, *Babesia*, or *Cytauxzoon*, respectively. Of the 36 *Babesia*-positives, 16 (44%) were from *A. americanum*, 19 (53%) were from *D. variabilis* and one (3%) was from an *I. scapularis*. Importantly, nine *Babesia*-positive ticks were removed from humans in Georgia (n=2), Kentucky (n=1), Pennsylvania (n=1), and Texas (n=5). Three *Babesia*-positive ticks were questing *A. americanum*, representing the first report of *Babesia* in Amblyomma spp. Six of the *Babesia*-positive *A. americanum* (one from a dog, two from feral hogs, and three from humans) were *Babesia* sp. Coco, a *Babesia* found in immunocompromised dogs. These data suggest that *A. americanum* might vector *Babesia* sp. Coco, although additional studies are warranted. Finally, these data highlight the usefulness of screening questing and fed ticks, as long as data interpretation stipulates that blood meals are present in non-questing ticks.

A project to characterize a previously detected novel *Babesia* species in Florida pumas was continued. Numerous studies were conducted to provide new biological, serologic, and molecular data on this *Babesia* species. Testing of over 900 bobcats from the eastern U.S. suggests that this *Babesia* is restricted to southern Florida and pumas. No evidence of congenital infection in kittens (n=24) of *Babesia*-infected females was noted. Genetic analyses revealed high diversity in the ITS1 rRNA region, which suggests that the parasite is endemic. Serologic cross-reactivity (1:256) was noted between serum from *Babesia*-infected pumas and *B. odocoilei*, *B. canis*, and *B. bovis* antigens. Ectoparasite surveys conducted for the past 18 years on Florida pumas indicate that *Dermacentor variabilis* (81% of all pumas with ticks; mean 6 ticks/infested puma) and *Ixodes scapularis* (75% of pumas with ticks; mean 13 ticks) were common.

Ehrlichiosis and Other Tick-Borne Bacterial Pathogens

A project to determine the effects of long-term prescribed fire management regimes on ticks and tick-borne pathogen prevalence was continued. This project is primarily funded by a CDC/UGA Collaborative Grant. Additional support has come from the J.W. Jones Research Center at Ichauway and Warnell School of Forestry and Natural Resources, and SCWDS. Goals for the project include 1) determining seasonality and tick species composition of ticks in southwest Georgia; 2) comparing effects of various burn regimes (burn time-interval, burn intensity and burn regime of surrounding land) on tick abundance, species composition and pathogen prevalence. We aim to perform these comparisons while accounting for other factors known to affect tick abundance, such as host abundance, vegetation structure, and microclimatic conditions; and 3) use GIS to extrapolate and map potential disease risk to humans in Georgia based on vegetation structure and presence of burns.

Field work on the tick-fire project at the J.W. Jones Ecological Research Center at Ichauway was completed December 2011, but laboratory testing of ticks and data analysis were conducted during this fiscal year. Three manuscripts are in preparation, and a manuscript on the experimental effects of fire ants on survival of ticks was published in the *Journal of Medical Entomology*.

Baylisascaris procyonis

A project to determine the distribution of *Baylisascaris procyonis* infection in raccoons and other potential hosts was continued.

A study to determine the distribution of *B. procyonis* in North Carolina was completed, and a manuscript was published in *Parasitology Research.* The project to determine the distribution of *B. procyonis* in Florida was continued. The Florida Fish and Wildlife Conservation Commission continued to submit raccoons, intestines, and worm samples to SCWDS for testing. Samples

have been preserved for future confirmation. In addition, serum samples from wildlife rehabilitators from Florida were tested for antibodies to *B. procyonis*. Two of 53 individuals had antibodies indicating past or current infection.

Trypanosoma cruzi

The study to investigate the molecular and biological characteristics of *Trypanosoma cruzi* in the United States was continued. *T. cruzi* can cause fatal disease (Chagas disease) in humans and a number of other mammalian species, most commonly the domestic dog. Although human cases are common in Latin America, few human cases are reported in the United States; however, a number of fatal canine and exotic animal cases are reported annually. This study is funded by the National Institutes of Health.

During this period, data analysis continued and several manuscripts were initiated. Isolates of *T. cruzi* were provided to collaborators and other researchers interested in *T. cruzi* in the United States.

Mycoplasma and Parasites of Gopher Tortoises

The study to assess the health of select gopher tortoise populations in Georgia was continued in an effort to better understand the role of *Mycoplasma* on population morbidity and mortality. This project is a collaborative effort between the J.W. Jones Ecological Research Center (JERC) in Newton, Georgia, the University of Georgia's D.B. Warnell School of Forestry and Natural Resources, and SCWDS. The project has been partially funded by the JERC, St. Catherine's Island Foundation, Gopher Tortoise Council, Sigma Xi, the Roger Williams Park Zoo, and the Morris Animal Foundation.

A primary aim of this project is to determine the prevalence of *Mycoplasma*-related upper respiratory tract disease (URTD) in select tortoise populations throughout Georgia. We found that exposure to both *Mycoplasma agassizii* and *M. testudineum*, pathogens associated with URTD, varied geographically among 11 tested Georgia tortoise populations. The prevalence of antibodies to *M. agassizii* in individual populations was either very low (0-3%, n=7 populations) or very high (96- 100%, n=4 populations), whereas there was variation in the prevalence of antibodies to *M. testudineum* among populations (38%-61%), with only one site being negative. Five sites were seropositive for both pathogens, and these were the only sites where we observed tortoises with clinical signs consistent with URTD. Interestingly, we did not find tortoises with clinical signs of URTD at sites with tortoises that were seropositive only for *M. testudineum*, which provides evidence that this organism may be of limited pathogenicity for gopher tortoises. Collectively, these data indicate that both *M. agassizii* and *M. testudineum* are present in Georgia populations of gopher tortoises and that clinical disease is apparent in populations where both pathogens are present.

This year, we finished a project aimed at investigating the effects of URTD on the behavior of gopher tortoises. We radio-tracked thirty tortoises (16 adult males, 14 adult females) from an area of the Jones Center (called GG) to compare home range size to that of a previous study and monitored carapace temperature of these tortoises using data loggers to determine thermoregulatory behavior. An additional 10 adult tortoises (6 males and 4 females) with severe clinical signs of URTD from elsewhere on the Jones Center property were monitored for comparison with current data from tortoises from GG that were asymptomatic or had only mild symptoms of URTD. We found no significant difference in 95% minimum convex polygon (MCP) home range size between the GG 'asymptomatic' and 'mild' tortoises (mean 1.38ha). Home ranges of 'severe' tortoises were significantly larger (mean 166.75 ha) than asymptomatic and mild tortoises ($F=5.60$, $df=2$, $p=0.0081$). Severely affected tortoises moved long distances

over short periods of time, contradicting the hypothesis that chronically infected tortoises are less likely to emigrate. Prevalence of *M. agassizii* antibodies was similar among the three groups (98% overall), but prevalence of *M. testudineum* was lower in the asymptomatic (7%) and mild (14%) groups compared with the severely affected group (50%) (p=0.0019). Variation in the average carapacial temperatures of severely affected tortoises varied significantly from temperatures of mild and asymptomatic tortoises (H=17.142, df=2, p=0.0002), which suggests there were differences in thermoregulatory behavior of severely ill tortoises. Our 15-year recapture data from GG suggests that, despite high seroprevalence, population density can remain constant over time. However, emigration of these animals, especially tortoises with clinical disease, may play an important role in dispersal and persistence of pathogens.

We continued a study on a hemogregarine parasite of gopher tortoises in an effort to understand the natural history of the parasite, especially any association with *Amblyomma tuberculatum*, the gopher tortoise tick. Four of six sampled populations were infested with *A. tuberculatum* with infestation rates ranging from 13-100%. All of these tick-positive sites were also positive for haemogregarines, but prevalence varied greatly from 6% to 86%. Tortoises from the two tick-negative sites were all negative for haemogregarines. The prevalence of haemogregarines was significantly higher in populations that had significantly higher infestation rates for *A. tuberculatum*. Across all of the sites, parasitemias were low and ranged from 0.01-7%.

White Nose Syndrome

SCWDS continued surveillance for white nose syndrome (WNS) and its causative agent, *Geomyces destructans*, in the Southeast. We examined 159 bats of 13 species for WNS and *G. destructans*. WNS was confirmed in tri-colored bats (*Perimyotis subflavus*), little brown bats (*Myotis lucifugus*), northern long-eared bats (*Myotis septentrionalis*), an eastern small-footed bat (*Myotis leibii*), and a federally endangered Indiana bat (*Myotis sodalis*). The eastern small-footed bat represents the first of its species confirmed WNS positive at SCWDS, although other individuals had been previously confirmed elsewhere. *Geomyces destructans* DNA, although not confirmed histologically, was found on two additional federally endangered species, gray bats (*Myotis griescens*) and a Virginia big-eared bat (*Corynorhinus townsendii virginianus*), as well as big brown bats (*Eptesicus fuscus*). No evidence of WNS was found on any southeastern myotis (*Myotis austroriparius*), evening bat (*Nycticeius humeralis*), Brazilian free-tailed bat (*Tadarida brasiliensis*), eastern red bat (*Lasiurus borealis*), or Seminole bat (*Lasiurus seminolus*) submitted. WNS was confirmed in bats from Alabama, Georgia, Kentucky, North Carolina, South Carolina, Tennessee, and Virginia, but not in bats submitted from Arkansas, Florida, Maryland, Louisiana, and New Jersey. WNS was diagnosed in Georgia and South Carolina, as well as Mammoth Cave National Park, Cumberland Gap National Historical Park, and Fern Cave National Wildlife Refuge for the first time.

Additional *G. destructans* spore isolates were prepared from the cultures of each WNS positive case. These isolates can be used to determine if there were any variations in the fungus as it spreads geographically and among various species. Fungal growth on culture has been successful and isolates grown at SCWDS have been utilized in collaboration with various researchers to analyze growth factors and enzyme activity of *G. destructans*. Multiple commercially available antifungal agents and water clarifiers were evaluated for their potential use as control agents for *G. destructans*.

The work determining the potential for fungal spores or hyphae to remain viable on fabric was continued. Laboratory based trials were conducted comparing *G. destructans* persistence on various common fabrics under variable environmental conditions. Laboratory based studies

are ongoing including *in vitro* assays using patagial explants from big brown bats and evaluation of the utility of various commercially available swab types for their use in WNS surveillance.

Dr. Lisa Last serves on the WNS Diagnostic Working Group to provide guidance in the formulation of the National Plan for Assisting States, Federal Agencies, and Tribes in Managing White-Nose Syndrome in Bats.

Salmonella in Wildlife

A study funded by a no-cost extension with the NIH and in collaboration with the UGA College of Environmental Health to investigate *Salmonella* in wildlife in two watersheds (one in north Georgia, one in south Georgia) continues. Worldwide and domestically, the rising popularity of pet reptiles is linked with increasing *Salmonella* infections. In 2005, roughly 93,000 cases were linked to reptiles – and the number of cases is still growing. The CDC reports on a multistate outbreak of reptile-associated salmonellosis linked to turtles in which 371 cases were reported in 2013. Of those, 90% were confirmed to have had interactions with turtles. The majority of the people involved in these cases purchased turtles from street vendors.

In response to this multi-state outbreak of pet reptile-associated human salmonellosis and because there is general lack of knowledge about *Salmonella* prevalence in wild, aquatic turtles, particularly in the southeast where turtles are harvested for food and exportation to foreign food markets, our study investigates the prevalence of *Salmonella* infection in six species of southeastern wild aquatic turtles.

We continued capturing aquatic turtles from eight small man-made ponds within Athens-Clarke County in Athens, Georgia, and six aquatic turtle species were caught from May to July 2013. The species captured and sampled were the common snapping turtle (*Chelydra serpentine*), common musk turtle (*Sternotherus odoratus*), painted turtle (*Chrysemys picta*), pond slider (*Trachemys scripta*), spiny softshell turtle (*Apalone spinifera*), and loggerhead musk turtle (*Sternotherus minor*). Feces were collected, isolation of *Salmonella* followed standard methodology, and *Salmonella* isolates were forwarded to the APHIS National Veterinary Services Laboratory for serotyping.

To date, prevalence of infection is as follows: spiny softshell turtle, 100% (4/4); eastern painted turtle, 15% (10/64), yellow-bellied slider, 14% (7/49), common musk turtle, 19% (9/47), common snapper, 40% (8/20) and loggerhead musk turtle, 43% (3/7). Bottom-dwelling, non-basking turtles had a higher prevalence than baskers. There was no statistically significant difference in the prevalence of infection by site of collection. Juvenile painted turtles and pond sliders were shedding *Salmonella* at a higher rate than adults, but there were no differences by gender or in any of the other species examined. The month with the highest prevalence is July, although no sampling was achieved in August. Identification of all serotypes is pending; however, some serotypes identified to date have been previously associated with human salmonellosis.

White Ibis Health

The white ibis (*Eudocimus albus*), a wading bird synonymous with the Florida Everglades, is in decline. A portion of the population has become heavily dependent on urbanized wetlands, where the perception is that they are abundant and healthy. The project we are conducting is designed to estimate the health risks that white ibises pose for people or other wildlife by determining the prevalence and serotypes of *Salmonella* spp. and other pathogens they carry, and to identify health risks for white ibises associated with this shift in habitat utilization by determining the health status of urbanized white ibises.

From July 1, 2012-July 2013, we continued our health assessment of urbanized white ibis in three ways: 1) we studied the prevalence of *Salmonella* spp. of white ibis nestlings in a gradient of distance from the Everglades to areas of anthropogenic foraging zones for parents rearing them; 2) continued to capture adult white ibises in urban parks to measure their health and pathogen prevalence and utilized fecal and plasma corticosterone levels and bacterial killing assays to assess their stress and immune function, and 3) captured adults in urban parks and outfitted them with radio transmitters and unique color bands to determine the proportion of time that ibises utilize urban parks. Analyses and results are pending.

Feral Swine Studies

SCWDS previously developed and activated the National Feral Swine Mapping System (NFSMS) through a Cooperative Agreement with USDA-APHIS-Veterinary Services. The NFSMS is an internet-based data collection system being used to collect and display current data on the distribution of feral swine in the United States. GIS development and programming support was conducted in collaboration with the Center for Remote Sensing and Mapping Science, Department of Geography, and Information Technology, College of Veterinary Medicine, The University of Georgia.

Previous feral swine distribution maps produced by SCWDS during 1982, 1988, and 2004 were developed through individual contacts with each state wildlife management agency, and a single final map was completed for each year. The NFSMS is used to collect data from state wildlife management agencies and other state/federal wildlife and agriculture agencies via a website. Distribution data can be submitted by agency personnel at any time; these data are evaluated on a continual basis, and the distribution map is updated with verified additions on a monthly basis. Feral swine populations and/or sightings are designated on the map either as established breeding populations or as sightings. The NFSMS is accessed via the internet at http://www.feralswinemap.org/ and will be maintained by SCWDS through further Cooperative Agreements with USDA-APHIS-Veterinary Services.

SCWDS personnel are participating in several working groups related to feral hogs including Georgia Feral Hog Working Group; and the Feral Hog Community of Practice, Extension, Land-Grant University System to address issues associated with feral swine. Dr. Joseph Corn is Chair of the USAHA Feral Swine Subcommittee on Brucellosis and Pseudorabies.

Endangered Key Deer Studies

SCWDS completed a three-year study funded by the U.S. Fish and Wildlife Service to assess the risk of environmental transmission of *Mycobacterium avium paratuberculosis* (MAP) for the endangered Florida Key deer. Field studies were conducted from 2009 through 2011. Field collections included collection of data on areas of fresh water, artificial water supplies, and artificial feeding sites. In addition, environmental specimens were collected from areas of fresh water and artificial water supplies, tissue specimens were collected from road-killed Key deer, and fecal specimens were collected from the ground. Testing for MAP was conducted at the Johnes Information Center, University of Wisconsin, and at SCWDS. MAP was isolated from 36/369 (10%) fecal pellet samples collected throughout the Key deer range on Big Pine Key and the Newfound Harbor Keys and all of the positive samples (36/142 (25%) were from Little Palm Island): 3/43 (7%) necropsied Key deer (3/3 (100%) from Little Palm Island); and 1/729 (0.1%) environmental samples (1/81 (1%) from Little Palm Island). Of the three MAP-positive Key deer, pooled tissue samples from the ileum, cecum, and ileocecal lymph node from two were MAP-positive; feces from one of these was culture positive. The third deer was MAP-positive by PCR. A manuscript was prepared and is in review.

National Fish and Wildlife Health Initiative

SCWDS has been deeply involved in the development of a National Fish and Wildlife Health Initiative (NFWHI) since it was first discussed in the spring of 2005. Dr. John Fischer served as Vice Chair of the Association of Fish and Wildlife Agencies' (AFWA) Working Group drafting the NFWHI and now serves as Vice Chair of the NFWHI Steering Committee. 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 of 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. Dr. John Fischer will serve as the Vice Chair of the NFWHI Steering Committee and is assisting in the repopulation of this leadership group.

One Health - APHIS Veterinary Services

Dr. John Fischer attends regular One Health meetings at the offices of APHIS in Conyers, Georgia, and presented SCWDS One Health activities at one meeting. In addition to APHIS employees, the meetings are attended by personnel from the Georgia Departments of Agriculture and Public Health, the U.S. Centers for Disease Control and Prevention, Zoo Atlanta, The University of Georgia, and others. In June 2013, Dr. Joseph Corn provided a presentation regarding the national distribution and health issues associated with feral swine to this group.

Informational Activities

Since July 1, 2012, 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 SCWDS BRIEFS were prepared and distributed in hard-copy format to more than 2,150 individuals worldwide and via electronic copy to more than 375 individuals and organizations. Many newsletter recipients now use on-line access to download and print copies rather than receive a paper copy via mail. 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.

SCWDS has continued to update and modify the web page (www.scwds.org). The website format was dramatically changed this year, but it still 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

The educational opportunities in the field of wildlife health are not available at other universities to the extent offered by SCWDS. Thus, SCWDS has a strong 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 receive degrees in disciplines such as Veterinary Sciences, Infectious Diseases, or Veterinary Pathology.

Dr. Michael Yabsley offered a Wildlife Disease Ecology and Management course (WILD/POPH 5100/7100) through the D.B. Warnell School of Forestry and Natural Resources. This course is open to both undergraduate and graduate students in Spring 2013. In addition, Dr. Yabsley taught a Maymester course on Field Methods in Wildlife Research, Management, and Disease Monitoring Methodologies (FORS 4600/6600) during Maymester 2013. Drs. Yabsley and Stallknecht taught a Wildlife Health seminar (POPH 8580) in Fall 2012 and Spring 2013. SCWDS has continued to provide educational opportunities for veterinary students at UGA; in 2013, Dr. Stallknecht taught a Wildlife Health Elective (IDIS 5900) and Veterinary Epidemiology (IDIS 5250); Dr. Yabsley, with other faculty from the Department of Infectious Diseases, co-taught Veterinary Helminthology, Ectoparasitology, and Protozoology; and Dr. Sonia M. Hernandez taught POPH 5410, a veterinary externship program open to 3rd and 4th year veterinary students. Dr. Hernandez offered Wildlife Disease Ecology: Investigation and Management (WILD8990) to graduate students in Fall 2012. Dr. Hernandez was course coordinator for a 6 credit month-long study abroad program in Costa Rica called Conservation Medicine/Conservation Biology (POPH 5118) during Summer 2012. Dr. Yabsley served as co-instructor. In Spring 2013, Dr. Hernandez was co-instructor for Ornithology (WILD 4060/6060) for which she taught half of the lectures and labs. Drs. Yabsley and Hernandez also each taught a Freshman Odyssey Seminar in Fall 2012 and they were called "Wildlife Parasites: Their Role in Public Health" (Yabsley) and "So You Want to be a Veterinarian?" (Hernandez). 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 University of Georgia's (UGA) College of Veterinary Medicine and externships for senior students from other veterinary colleges. These rotations enable students to work with wildlife health professionals in a variety of capacities. Students generally are involved in field

collections of biological samples; they may assist with laboratory research, and they help conduct necropsies on diagnostic accessions. Dr. Hernandez coordinated the program and was assisted by Drs. Ballard, Brown, Munk, and Nemeth, others at SCWDS who provided instruction and guidance for the 14 veterinary students who enrolled in wildlife health externships. Students came from the veterinary colleges at Atlantic Veterinary College, Iowa State University, North Carolina State University, Ohio State University, Pennsylvania State University, Western College, and the universities of Calgary, Georgia, Illinois, Minnesota, and Wisconsin.

Graduate Studies

SCWDS offers graduate degree programs focusing on wildlife health related research in both the College of Veterinary Medicine and the D.B. Warnell School of Forestry and Natural Resources. This graduate instruction effort includes close collaboration with faculty strictly affiliated with SCWDS and faculty with wildlife health expertise within other academic departments in the College of Veterinary Medicine. During FY 2013, the following 45 graduate students were affiliated with SCWDS faculty:

Dr. Brown served on the Graduate Committees of five PhD students (Jennifer Ballard - Veterinary Sciences; Andy Ramey and Rebecca Poulson - Department of Infectious Diseases and Department of Population Health, Monique Franca and Jusun Hwang - Department of Pathology, College of Veterinary Medicine) and one MS student (Jesse Thomas - D.B. Warnell School of Forestry and Natural Resources).

Dr. John R. Fischer served as Major Professor for one PhD student (Jennifer Ballard - Veterinary Sciences, College of Veterinary Medicine, and on the Graduate Committees for Bradley Cohen (PhD student D.B. Warnell School of Forestry and Natural Resources) and Emily Belser (MS student D.B. Warnell School of Forestry and Natural Resources).

Dr. Sonia M. Hernandez served as Major Professor for three PhD students (April Conway and Albert Mercurio - D.B. Warnell School of Forestry and Natural Resources, and Kristy Segal - Odum School of Ecology), three MS students (Gabrielle Robinson, Catie Welch, and Shannon Curry - D.B. Warnell School of Forestry and Natural Resources) and one Master of Natural Resources student (Viviana Gonzalez - D.B. Warnell School of Forestry and Natural Resources). Dr. Hernandez also served on the Graduate Committees of four PhD students (Julie Rushmore - Odum School of Ecology; Jessica Gonynor-McGuire, Ana Joy, and Barbara Shock - D.B. Warnell School of Forestry and Natural Resources).

Dr. Mead served as the Major Professor for one UGA M.S. student (Christopher Cleveland - Veterinary and Biomedical Sciences, College of Veterinary Medicine). Dr. Daniel Mead also served on the Graduate Committees of two UGA PhD students (Jennifer Ballard - Veterinary and Biomedical Sciences, College of Veterinary Medicine and Sarah Bowden - Odum School of Ecology), one UGA D.V.M./M.P.H student (Lydia Young - College of Public Health and College of Veterinary Medicine), and two UGA M.S. students (T.J. McGaha - Department of Entomology and Erica Teasley - Odom School of Ecology). Additionally, Dr. Mead served on the Graduate Committee of one Emory University PhD student (Rebecca Levine - Department of Environmental Studies).

Dr. David E. Stallknecht served as Major Professor for two PhD students (Andy Ramey and Rebecca Poulson - Department of Infectious Diseases and Department of Population Health, College of Veterinary Medicine). He also served on the Graduate Committees of five PhD students (Monique Franca and Jennifer Ballard - Department of Pathology, College of Veterinary Medicine; Whitney Kistler and Barbara Shock - D.B. Warnell School of Forestry and

Natural Resources) and one MS student (Morgan Slusher - D.B. Warnell School of Forestry and Natural Resources).

Dr. Michael J. Yabsley served as Major Professor for six MS students (Heidi Murray, Morgan Slusher, Kimberly Sonderman, Kimberly McDermid, Sarah Coker, and Jessie Thomas - D.B. Warnell School of Forestry and Natural Resources) and five PhD students (Elizabeth Gleim, Jessica Gonynor-McGuire, Whitney Kistler, Barbara Shock, and Todd Nims - D.B. Warnell School of Forestry and Natural Resources). Dr. Yabsley served on the Graduate Committees of four PhD students (Albert Mercurio - D.B. Warnell School of Forestry and Natural Resources and Julie Rushmore, Kristy Segal, and Jamie Winternitz - Odum School of Ecology). Dr. Yabsley also served as Faculty Research Mentor for one Merck-Merial Veterinary Scholar student (Holly Burchfield), eight undergraduate students (John Rossow, Krysta Janas, Brianna Williams, Mireya Smith, Lauren Lipscei, Rachel Lock, Joyce Huang, Scarlett Sumner), one REU Ecology of Infectious Diseases (UGA) student (Candace Cooper), and a Young-Scholars high school student (Madeline Mullen).

FUNDING CONTRIBUTIONS BY NON-STATE SCWDS COOPERATORS AND OTHER SOURCES

USDI Wildlife Disease Problems

(USDI Proposal #G11AC20003)

Major Investigators: John R. Fischer, Daniel G. Mead, and Michael J. Yabsley

This project began January 1, 2013, and is scheduled to terminate December 31, 2013.

The major objectives are: (1) to provide assistance to the U.S. Department of the Interior, State Fish and Wildlife Agencies, and other governmental agencies on disease problems in wildlife; (2) to determine the significance of health problems for wildlife populations and the potential for spread to other species, including domestic animals and humans; (3) to investigate and recommend sound wildlife management practices that will aid in disease prevention and control; and (4) to disseminate information from wildlife disease investigations through proper channels for use in wildlife management and public relations.

Specific activities emphasized by the SCWDS Steering Committee for the period of this contract are to:

- 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 agencies that support SCWDS, services will be provided to state and federal animal health authorities, public health officials, wildlife researchers and managers, and other organizations when it is in the interests of wildlife conservation and SCWDS resources are available.
- Prepare informational materials on salient wildlife diseases to increase understanding of disease biology among wildlife managers and to help wildlife agencies communicate with the public and the news media.
- Provide disease evaluation services on translocated wildlife in order to assess potentials for introduction and dissemination of diseases and parasites to existing populations of wildlife, domestic livestock, poultry, or humans.
- Monitor selected wildlife diseases to determine their impact on the 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 and federal 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.

NIAID Centers of Excellence for Influenza Research and Surveillance

(NIH, University of Minnesota #033077-01, Subaward #P002477002)

Major Investigators: David E. Stallknecht, Daniel G. Mead, Elizabeth W. Howerth, and Mark Tompkins

This project began March 30, 2007, and is scheduled to terminate March 29, 2014.

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.

Relationships Involving Wildlife, Livestock, and Poultry

(USDA-APHIS-Veterinary Services Cooperative Agreement #13-9100-1407-CA)

Major Investigators: John R. Fischer, Joseph L. Corn, and Daniel G. Mead

This project began April 1, 2013, and is scheduled to terminate March 31, 2014.

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 assist APHIS in preparation and surveillance for foreign and emerging animal diseases that threatens domestic and wild animals.
- To develop and disseminate information regarding diseases transmissible between wild animals, domestic livestock, poultry and humans.
- To serve APHIS in an advisory capacity on wildlife management and its relationship to diseases of domestic animals.
- To act as liaison among state, federal, and private sectors responsible for the health and well-being of this nation's domestic livestock, poultry, and wildlife resources.

Exotic Arthropod Surveillance

(USDA-APHIS-Veterinary Services, Cooperative Agreement #13-9100-1407-CA)

Major Investigators: Joseph L. Corn and John R. Fischer

This project began April 1, 2013, and is scheduled to terminate March 31, 2014.

The goal of the program is to provide for surveillance and early detection of exotic ticks in south Texas and surveillance for *Culicoides* spp. associated with wildlife in the southeastern United States.

The objectives of this project are:

- To conduct surveillance for exotic ticks and other livestock arthropods associated with wildlife in Texas.
- To conduct surveillance for *Culicoides* in the southeastern United States.
- To conduct surveillance for exotic ticks and other livestock arthropods on wildlife in Florida and possible additional southeastern States.
- To conduct additional surveillance for arthropods in the southeastern United States including in response to introductions of foreign arthropod-borne diseases as per request by USDA-APHIS-VS.
- To assist in the development of control measures where exotic tick and livestock arthropod eradication is deemed necessary.
- To provide technical support to determine the role of wildlife in the maintenance and dissemination of ticks and livestock arthropods.

National Feral Swine Mapping System

(USDA-APHIS-Veterinary Services, Cooperative Agreement #13-9100-1407-CA)

Major Investigators: Joseph L. Corn and John R. Fischer

This project began April 1, 2013, and is scheduled to terminate March 31, 2014.

The objectives of this project are:

- To maintain the National Feral Swine Mapping System, an internet based system for collection of real time data on the distribution of feral swine in the United States.
- To provide distribution data for feral swine in the United States to state and federal agencies and military installations.
- To collaborate on working groups addressing feral swine issues and give presentations of feral issues to state and federal agency personnel.

Diagnostic, Field, and Training Assistance for Avian Health and Disease Monitoring

(USFWS Cooperative Agreement #91200-1-9711)

Major Investigators: John R. Fischer and Justin D. Brown

This project began May 12, 2011, and is scheduled to terminate April 30, 2014.

The goal of this program is to support the avian conservation, surveillance, and management goals of the U.S. Fish and Wildlife by encompassing disease and health issues, including all migratory bird species.

The objectives of this project are:

- To perform examinations and diagnostic testing on an as needed basis in response to mortality events or project needs including gross and microscopic pathology, microbiology, parasitology, toxicology, and other diagnostic procedures as necessary.
- To conduct cooperative investigation of diseases impacting avian populations; assist in collection and processing of biological specimens for FWS Avian Health and Disease Program regional research projects; and assist Refuges, FWC field stations, and Avian Health and Disease Program Regional Coordinators during mortality events and surveillance projects.

- To assist in providing avian health and disease outreach activities to Refuges, FWS field stations; assist in organizing and presenting regional and national training courses; provide educational materials for courses, fact sheets, and web communications.

Diagnostic, Field, and Training Assistance for Avian Health and Disease Monitoring

(USFWS Cooperative Agreement #F13PX01403)

Major Investigators: John R. Fischer and Justin D. Brown

This project began August 1, 2012, and is scheduled to terminate July 31, 2015.

The goal of this program is to establish 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 to include 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.

Inter-Hemispheric Transport of Avian Paramyxoviruses by Migratory Birds

(USGS Alaska Science Center, Grant #G12PX01129)

Major Investigators: David E. Stallknecht

This project began September 1, 2012, and is scheduled to terminate September 30, 2013.

The objective of this project is to investigate the inter-hemispheric transport of avian influenza viruses by migratory birds. SCWDS will be conducting virus isolations on cloacal and fecal swab samples collected from a variety of waterbirds sampled along the Texas Gulf Coast in February and March of 2013.

Experimental Challenge of American Common Eiders (*Somateria mollissima dresseri*) with Wellfleet Bay Virus by Multiple Routes of Inoculation

(USGS National Wildlife Health Center, USFWS, Grant #F12AP00979)

Major Investigators: John R. Fischer

This project began August 1, 2012, and is scheduled to terminate July 31, 2014.

The research objective of this project is to determine the likely route(s) of transmission of Wellfleet Bay Virus among free-ranging common eiders by comparing routes of inoculation in a controlled setting.

Risk of Environmental Transmission of Johne's Disease for Endangered Florida Key Deer

U.S. Fish and Wildlife Grant #F09AP00057

Major Investigators: Joseph L. Corn

This project began September 1, 2009, and is scheduled to terminate December 31, 2013.

The objectives of this agreement are:

- To identify fresh water sites, artificial water supplies, and artificial feeding sites used by Key deer within the known distribution of *Mycobacterium avium* subspecies *paratuberculosis* (*Map*)-infected Key Deer.
- To determine if *Map* contamination of fresh water sites, artificial water supplies and/or artificial feeding sites occurs and if these sites can serve as sources for transmission.
- To determine if *Map* in Key deer has spread from the initial focus in the Newfound Harbor subpopulation into the main population of Key deer in Big Pine Key.
- To determine parameters for age-structure, reproductive performance and nutritional condition of Key deer and train National Key Deer refuge personnel to conduct a monitoring program.

Vector-Borne Disease Surveillance and Mosquito Diagnostic Support

(Chatham County Board of Commissions #P10-1-3-5)

Major Investigator: Daniel G. Mead

This project began May 22, 2009, and is scheduled to terminate June 30, 2014.

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

(DeKalb County Board of Health #13-823-C0001-11)

Major Investigator: Daniel G. Mead

This project began July 1, 2011, and terminated June 30, 2013.

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

USDA-APHIS-Veterinary Services Surveillance

(USDA Cooperative Agreement #12-9613-0032-CA)

Major Investigators: John R. Fischer, Joseph L. Corn, Daniel G. Mead, David E. Stallknecht, and M. Kevin Keel

This project began October 1, 2012, and terminated March 31, 2013.

The Cooperative Agreement was 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 were as follows:

- To assist APHIS in preparation and surveillance for foreign and emerging animal diseases that threatens domestic and wild animals.
- To develop and disseminate information regarding diseases transmissible between wild animals, domestic livestock, poultry and humans.
- To serve APHIS in an advisory capacity on wildlife management and its relationship to diseases of domestic animals.

- To act as liaison among state, federal, and private sectors responsible for the health and well-being of this nation's domestic livestock, poultry, and wildlife resources.

Exotic Arthropod Surveillance in the Southeastern United States and Puerto Rico

(USDA-APHIS Service Grant #12-9113-0808-CA)

Major Investigators: Joseph L. Corn and John R. Fischer

This project began April 1, 2012, and terminated May 31, 2013.

The goal of the program was to provide for surveillance and early detection of exotic ticks associated with wildlife in the southeastern United States and Puerto Rico.

The objectives of this project were:

- To conduct surveillance for exotic ticks and other livestock arthropods associated with wildlife in selected geographic areas of the southeastern United States and Puerto Rico.
- To assist in the development of control measures where exotic tick and livestock arthropod eradication is deemed necessary.
- To conduct surveys for ticks and livestock arthropods at selected sites as determined by USDA-APHIS-VS.
- To provide technical support to determine the role of wildlife in the maintenance and dissemination of ticks and livestock arthropods.

National Feral Swine Mapping System

(USDA-APHIS-Veterinary Services, Grant #12-9113-1156-CA)

Major Investigators: Joseph L. Corn and John R. Fischer

This project began April 1, 2012, and terminated March 31, 2013.

The objective of this project was to maintain the National Feral Swine Mapping System, an internet based system for collection of real time data on the distribution of feral swine in the United States.

USDA-APHIS-Wildlife Services Disease Training

(USDA Agreement #12-7100-0116-CA)

Major Investigators: John R. Fischer, Joseph L. Corn, M. Kevin Keel, and Michael J. Yabsley

This project began October 1, 2011, and terminated September 30, 2012.

The purpose of this project was to provide training to Wildlife Services (WS) personnel regarding foreign animal, zoonotic, and other diseases where wildlife may play a role in the epidemiology of the disease. This training included classroom, laboratory, and in-the-field training, and at a minimum, included the Emergency Animal Disease Preparedness Training Seminar held annually by SCWDS, and an annual wildlife disease training course specific to the kinds of wildlife or foreign animal disease-related activities in which Wildlife Services employees may be involved.

TECHNICAL PUBLICATIONS AUTHORIZED OR CO-AUTHORED BY SCWDS PERSONNEL

Published

Allison, A.B., D.J. Kohler, K.A. Fox, J.D. Brown, R.W. Gerhold, V.I. Shearn-Bochsler, E.J. Dubovi, C.R. Parrish, and E.C. Holmes. 2013. Frequent cross-species transmission of parvoviruses among diverse carnivore hosts. *Journal of Virology* 87(4): 2342-2347.

ABSTRACT: Although parvoviruses are commonly described in domestic carnivores, little is known about their biodiversity in nondomestic species. A phylogenetic analysis of VP2 gene sequences from puma, coyote, gray wolf, bobcat, raccoon, and striped skunk revealed two major groups related to either feline panleukopenia virus ("FPV-like") or canine parvovirus ("CPV-like"). Cross-species transmission was commonplace, with multiple introductions into each host species but, with the exception of raccoons, relatively little evidence for onward transmission in nondomestic species.

Armien, A.G., D.L. McRuer, M.G. Ruder, and A. Wunschmann. 2013. Purkinje cell heterotopy with cerebellar hypoplasia in two free-living American kestrels (*Falco sparverius*). *Veterinary Pathology* 50(1): 182-187.

ABSTRACT: Two wild fledgling kestrels exhibited lack of motor coordination, postural reaction deficits, and abnormal proprioception. At necropsy, the cerebellum and brainstem were markedly underdeveloped. Microscopically, there was Purkinje cells heterotopy, abnormal circuitry, and hypoplasia with defective foliation. Heterotopic neurons were identified as immature Purkinje cells by their size, location, immunoreactivity for calbidin D-28 K, and ultrastructural features. The authors suggest that this cerebellar abnormality was likely due to a disruption of molecular mechanisms that dictate Purkinje cell migration, placement, and maturation in early embryonic development. The etiology of this condition remains undetermined. Congenital central nervous system disorders have rarely been reported in birds.

Berry, B.S., K. Magori, A.C. Perofsky, D.E. Stallknecht, and A.W. Park. 2013. Wetland cover dynamics drive hemorrhagic disease patterns in white-tailed deer in the United States. *Journal of Wildlife Diseases* 49(3): 501-509.

ABSTRACT: While vector-borne diseases are known to be particularly influenced by environmental factors, the impact of land-cover change on vector-borne wildlife disease patterns is poorly understood, largely due to the paucity of data on disease occurrence at extensive spatial and temporal scales. Widespread and rapid anthropogenic land-cover change, especially urbanization, has transformed the U.S. landscape during the last century. Epizootic hemorrhagic disease virus and bluetongue virus, vectored by *Culicoides* biting midges, are two RNA viruses in the *Orbivirus* genus that cause severe hemorrhagic disease (HD) in white-tailed deer (*Odocoileus virginianus*). We examined the spatial dynamics of HD affecting white-tailed deer in the contiguous United States in two periods covering 1980 to 2007 in connection with land-cover change over the same time. Using spatial statistical modeling, wetland cover emerges as a critical driver of HD morbidity, whereas the drivers of mortality patterns are more complex. Increasing wetland cover is positively associated with HD morbidity, which is consistent with the ecologic requirements of the *Culicoides* vector. Wetland cover is inherently dynamic due to its importance to biodiversity and water quality

as well as its utility for other purposes when drained. Accordingly this analysis helps in understanding the consequences of changing wetlands on vector-borne disease patterns, to identify disease hotspots in a large landscape, and to forecast the spatial spread of HD and related diseases.

Brown, J.D., and D.E. Stallknecht. 2013. Wild bird surveillance for avian influenza virus. Book chapter in *Methods in Molecular Biology*, 2nd Edition, pp. 85-98. E. Spackman (Editor). Humana Press, Totowa, New Jersey.

Brown, J.D., R.D. Berghaus, T.P. Costa, R. Poulson, D.L. Carter, C. Lebarbenchon, and D.E. Stallknecht. 2012. Intestinal excretion of a wild bird-origin H3N8 low pathogenic avian influenza virus in mallards (*Anas platyrhynchos*). *Journal of Wildlife Diseases* 48(4): 991-998.

ABSTRACT: Mallards (*Anas platyrhynchos*) and other dabbling ducks in the genus *Anas* are an important component of the wild bird reservoir for avian influenza (AI) virus; these viruses are maintained in migratory duck populations through a fecal-oral transmission route. We provide a detailed characterization of intestinal viral shedding in mallards infected with a wild bird-origin low pathogenic (LP) AI virus. Five of eight, one-month-old mallards inoculated with a high dose of an H3N8 LP AI virus became infected as determined by reisolation and seroconversion. Infected birds excreted high concentrations of virus for up to 14 days postinoculation (DPI) without exhibiting overt clinical signs of disease. The pattern of viral shedding was relatively consistent between individual birds, with peak shedding on 2-3 DPI and a progressive decline over the remainder of infection. Detection of viral shedding varied depending on sample type (excrement sample or cloacal swab) and diagnostic test (virus isolation or real-time quantitative reverse transcription polymerase chain reaction). Our data provide detailed insights into the intestinal excretion of an H3N8 LP AI virus in mallards and the performance of diagnostic assays commonly used in wild bird surveillance. Such information is valuable for estimating potential risks for spillover of LP AI viruses from mallards to domestic animals, developing accurate transmission models for mallard populations and facilitating the interpretation and comparison of surveillance results from different studies.

Brown, J.D., R. Poulson, D.L. Carter, C. Lebarbenchon, M. Pantin-Jackwood, E. Spackman, E. Shepherd, M. Killian, and D.E. Stallknecht. 2012. Susceptibility of avian species to North American H13 low pathogenic avian influenza viruses. *Avian Diseases* 56(4s1): 969-975.

ABSTRACT: Gulls are widely recognized reservoirs for low pathogenic avian influenza (LPAI) viruses; however, the subtypes maintained in these populations and/or the transmission mechanisms involved are poorly understood. Although, a wide diversity of influenza viruses have been isolated from gulls, two hemagglutinin subtypes (H13 and H16) are rarely detected in other avian groups, and existing surveillance data suggests they are maintained almost exclusively within gull populations. In order to evaluate the host range of these gull-adapted influenza subtypes and to characterize viral infection in the gull host, we conducted a series of challenge experiments, with multiple North American strains of H13 LPAI virus in ring-billed gulls (*Larus delawarensis*), mallards (*Anas platyrhynchos*), chickens (*Gallus domesticus*), and turkeys (*Meleagris gallopavo*). The susceptibility to H13 LPAI viruses varied between species and viral strain. Gulls were highly susceptible to H13 LPAI virus infection and excreted virus via the oropharynx and cloaca for several days. The quantity and duration of shedding was similar between the two routes. Turkeys and ducks were resistant to infection with most strains of H13 LPAI virus, but low numbers of inoculated birds were infected after challenge with specific viral strains. Chickens were

refractory to infection with all strains of H13 LPAI virus they were challenged with. The experimental results presented herein are consistent with existing surveillance data on H13 LPAI viruses in birds, and indicate that influenza viruses of the H13 subtype are strongly host-adapted to gulls, but rare spill-over into aberrant hosts (i.e., turkeys and ducks) can occur.

Brown, J.D., R. Poulson, D.L. Carter, C. Lebarbenchon, and D.E. Stallknecht. 2013. Infectivity of avian influenza virus-positive field samples for mallards: what do our diagnostic results mean? *Journal of Wildlife Diseases* 49(1): 180-185.

ABSTRACT: Most surveillance programs for avian influenza (AI) virus in wild birds utilize molecular tests such as real-time reverse transcription-PCR (RRT-PCR) or virus isolation (VI) in embryonating chicken eggs. To provide insight into the relationship between positive diagnostic test results and infectivity for an avian host, we challenged mallards (*Anas platyrhynchos*) with mallard-derived cloacal swab field samples found positive by VI or RRT-PCR. Six of 11 samples that were both RRT-PCR positive and VI positive infected mallards. Sample infectivity for mallards appeared to be dependent on concentration of infectious virus in the sample; five of the six samples that replicated in mallards had a measurable virus titer, whereas four of the five samples that did not infect mallards had titer below the limit of detection (10(0.9) median embryo infectious dose/0.2 mL). None of seven samples that were RRT-PCR positive and VI negative infected mallards. These results indicate that embryonating chicken eggs are a sensitive diagnostic tool for detecting mallards excreting infectious AI virus at a high enough concentration to infect another mallard; however, not all cloacal swab field samples that are positive by VI or RRT-PCR are infective to another mallard. Additionally, our results indicate that mallards are susceptible to mallard-origin AI viruses that have not been propagated in embryonating chicken eggs and that some of these virus strains can infect birds at titers that are lower than those typically used in experimental challenge studies. These data highlight a need to examine the effects of using egg-propagated AI viruses in experimental trials.

Brown, V.L., J.M. Drake, D.E. Stallknecht, J.D. Brown, K. Pedersen, and P. Rohani. 2013. Dissecting a wildlife disease hotspot: the impact of multiple host species, environmental transmission and seasonality in migration, breeding, and mortality. *Journal of the Royal Society Interface* 10(79): 20120804.

ABSTRACT: Avian influenza viruses (AIVs) have been implicated in all human influenza pandemics in recent history. Despite this, surprisingly little is known about the mechanisms underlying the maintenance and spread of these viruses in their natural bird reservoirs. Surveillance has identified an AIV 'hotspot' in shorebirds at Delaware Bay, in which prevalence is estimated to exceed other monitored sites by an order of magnitude To better understand the factors that create an AIV hotspot, we developed and parametrized a mechanistic transmission model to study the simultaneous epizootiological impacts of multi-species transmission, seasonal breeding, host migration and mixed transmission routes. We scrutinized our model to examine the potential for an AIV hotspot to serve as a 'gateway' for the spread of novel viruses into North America. Our findings identify the conditions under which a novel influenza virus, if introduced into the system, could successfully invade and proliferate.

Chander, Y., N. Jindal, S. Sreevatsan, D.E. Stallknecht, and S.M. Goyal. 2012. Molecular and phylogenetic analysis of matrix gene of avian influenza viruses isolated from wild birds and live bird markets in the USA. *Influenza and Other Respiratory Viruses* doi: 10.1111/irv.12003.

ABSTRACT: Wild birds are the natural hosts for influenza A viruses (AIVs) and provide a niche for the maintenance of this virus. This study was undertaken to analyze nucleotide sequences of the matrix (M) gene of AIVs isolated from wild birds and live bird markets (LBMs) to index the changes occurring in this gene. M-gene of 229 avian influenza virus (AIV) isolates obtained from wild birds and LBMs was amplified and sequenced. Full-length sequences (~900 nt.) thus obtained were analyzed to identify changes that may be associated with resistance to adamantanes. Phylogenetic analysis of all sequences was performed using clustalW, and evolutionary distances were calculated by maximum composite likelihood method using mega (ver. 5.0) software. Twenty-seven different viral subtypes were represented with H3N8 being the most dominant subtype in wild birds and H7N2 being the predominant subtype among isolates from LBMs. Phylogenetic analysis of the M-gene showed a high degree of nucleotide sequence identity with U.S. isolates of AIVs but not with those of Asian or European lineage. While none of the isolates from wild birds had any antiviral resistance-associated mutations, 17 LBM isolates carried polymorphisms known to cause reduced susceptibility to antiviral drugs (adamantanes). Of these 17 isolates, 16 had S31N change and one isolate had V27A mutation. These results indicate independent evolution of M-gene in the absence of any antiviral drugs leading to mutations causing resistance indicating the need for continued active surveillance of AIVS.

Charles, R.A., S. Kjos, A.E. Ellis, J.C. Barnes, and M.J. Yabsley. 2013. Southern plains woodrats (*Neotoma micropus*) from southern Texas are important reservoirs of two genotypes of *Trypanosoma cruzi* and hosts of a putative novel *Trypanosoma* species. *Vector-Borne and Zoonotic Diseases* 13(1): 22-30.

ABSTRACT: *Trypanosoma cruzi,* the causative agent of Chagas' disease, is an important public health and veterinary pathogen. Although human cases are rare in the United States, infections in wildlife, and in some areas domestic dogs, are common. In 2008 and 2010, we investigated *T. cruzi* prevalence in possible vertebrate reservoirs in southern Texas, with an emphasis on southern plains woodrats (*Neotoma micropus*). Infection status was determined using a combination of culture isolation, polymerase chain reaction (PCR), and serologic testing. Based on PCR and/or culture, *T. cruzi* was detected in 35 of 104 (34%) woodrats, 3 of 4 (75%) striped skunks (*Mephitis mephitis*), 12 of 20 (60%) raccoons (*Procyon lotor*), and 5 of 28 (18%) other rodents including a hispid cotton rat (*Sigmodon hispidus*), rock squirrel (*Otospermophilus variegatus*), black rat (*Rattus rattus*), and two house mice (*Mus musculus*). Additionally, another *Trypanosoma* species was detected in 41 woodrats, of which 27 were co-infected with *T. cruzi*. Genetic characterization of *T. cruzi* revealed that raccoon, rock squirrel, and cotton rat isolates were genotype TcIV, while woodrats and skunks were infected with TcI and TcIV. Based on the Chagas Stat-Pak assay, antibodies were detected in 27 woodrats (26%), 13 raccoons (65%), 4 skunks (100%), and 5 other rodents (18%) (2 white-ankled mice [*Peromyscus pectoralis laceiaus*], 2 house mice, and a rock squirrel). Seroprevalence based on indirect immunofluorescence antibody testing was higher for both woodrats (37%) and raccoons (90%), compared with the Chagas Stat-Pak. This is the first report of *T. cruzi* in a hispis cotton rat, black rat, rock squirrel, and white-ankled mouse. These data indicate that based on culture and PCR testing, the prevalence of *T. cruzi* in woodrats is comparable with other common reservoirs (i.e., raccoons and opossums) in the United States. However, unlike raccoons and opossums, which tend to be infected with a particular genotype, southern plains woodrats were infected with TcI and TcIV at near equal frequencies.

Davis, A.K., A.C. Benz, L.E. Ruyle, W.M. Kistler, B.C. Shock, and M.J. Yabsley. 2013. Searching before it's too late: a survey of blood parasites in *Ctenosaura melanosterna*, a critically endangered reptile of Honduras. *ISRN Parasitology, Article ID 495304*, 6 pages.

ABSTRACT: For species at risk of extinction, any parasites they have would be expected to face a similar fate. In such cases, time is running out for efforts to identify and study their parasitic fauna before they are gone. We surveyed the hemoparasite fauna of 50 black-chested, spiny-tailed iguanas (*Ctenosaura melanosterna*), a critically-endangered species, on an island off the coast of Honduras. Blood samples from captured animals were tested for hemoparasites by thin blood smear and molecular analyses. Based on microscopy, two parasites were identified, a *Plasmodium* sp. In 14% of iguanas and a *Hepatozoon* sp. in 32%. For both parasites, parastemia levels were <0.1%. Prevalence and parasitemias of *Hepatozoon* declined with increasing host size, a pattern differing from most prior studies of saurian reptiles. From a subset of iguanas with microscopy-confirmed *Plasmodium* infections, sequence analysis of 454 bp of the cytochrome b gene indicated that the *Plasmodium* species was distinct from known *Plasmodium* and was most closely related to *P. chiricahuae* (96.5% similarity) followed by *P. mexicanum* (95.8% similarity). Efforts to amplify the *Hepatozoon* parasite using PCR were not successful. Additional surveys and studies of this host-parasite system would be valuable, both to science and to the management of this endangered animal.

Dorea, F.C., D.J. Cole, and D.E. Stallknecht. 2012. Quantitative exposure assessment of waterfowl hunters to avian influenza viruses. *Epidemiology and Infection* 15: 1-11.

ABSTRACT: The potential for direct transmission of type A influenza viruses from wild waterfowl to humans is undefined. This study estimated exposure of hunters to avian influenza virus (AIV) resulting from direct contact with potentially infected waterfowl in Georgia (GA), Louisiana (LA), and Minnesota (MN), and demonstrated variation in the risk of exposure to AIV by hunting location and time. Hunting begins earlier in MN, starting in October, and later in GA and LA, usually starting in November. In addition, the numbers of hunters and birds harvested varies considerably in each state, with LA hosting the largest harvest in the USA. Temporal effects resulted in variation of the exposure risk per hunter-day, with a higher risk associated with the earlier months of the hunting season. Exposure risk in locations varied due to AIV prevalence during each hunting season, average bird harvest per hunter-day, and ratio of juveniles/adult birds harvested (higher risk associated with higher ratios). Population risk is discussed based on the exposure risk and number of active hunters in each state per month. The risk of human exposure to AIV was also shown to be temporally distinct from the time of greatest risk of human influenza A infection during circulation of seasonal human influenza viruses, making recombination events due to co-infection unlikely.

Driskell, E.A., C.A. Jones, R.D. Berghaus, D.E. Stallknecht, E.W. Howerth, and S.M. Tompkins. 2012. Domestic cats are susceptible to infection with low pathogenic avian influenza viruses from shorebirds. *Veterinary Pathology* doi: 10.1177/0300985812452578.

ABSTRACT: Domestic cats are susceptible to infection with highly pathogenic avian influenza virus H5N1, resulting in pneumonia and in some cases, systemic spread with lesions in multiple organ systems. Recent transmission of the 2009 pandemic H1N1 influenza virus from humans to cats also resulted in severe pneumonia in cats. Data regarding the susceptibility of cats to other influenza viruses is minimal, especially regarding susceptibility to low pathogenic avian influenza viruses from wild birds, the reservoir host. In this study, the authors infected 5-month-old cats using two different North American shorebird avian influenza viruses (H1N9 and H6N4 subtypes), 3 cats per virus, with the goal of expanding the understanding of avian influenza virus infections in this species. These viruses replicated in inoculated cats based on virus isolation from the pharynx in 2 cats, virus isolation from the lung of 1 cat, and antigen presence in the lung via

immunohistochemistry in 2 cats. There was also seroconversion and lesions of patchy bronchointerstitial pneumonia in all of the cats. Infection in the cats did not result in clinical disease and led to variable pharyngeal viral shedding with only one of the viruses; virus was localized in the alveolar epithelium via immunohistochemistry. These findings demonstrate the capacity of wild bird influenza viruses to infect cats, and further investigation is warranted into the pathogenesis of these viruses in cats from both a veterinary medical and public health perspective.

Franca, M., R. Poulson, J.D. Brown, E.W. Howerth, R.D. Berghaus, D. Carter, and D.E. Stallknecht. 2012. Effect of different routes of inoculation on infectivity and viral shedding of LPAI viruses in mallards. *Avian Diseases* 56(4s1): 981-985.

ABSTRACT: We studied the effect of different routes of inoculation on the infectivity and duration of viral shedding in mallards (*Anas platyrhynchos*) infected with wild bird-origin low pathogenic avian influenza viruses (LPAIVs). One-month-old mallards were inoculated with 10^6 median embryo infectious doses in either A/mallard/MN/199106/99 (H3N8) or A/mallard/MN/35779/00 (H5N2) via 1 of 5 different routes: intranasal (IN), intratracheal (IT), intraocular (IO), intracloacal (IC), or intra-ingluvial (II). Birds in all routes of inoculation groups became infected with LPAIV as detected by virus isolation, real time reverse transcription polymerase chain reaction, and serology. Mallards in different route of inoculation groups had similar viral shedding through oropharynx and cloaca from 1 day postinoculation (dpi). The peak of oropharyngeal (OP) viral shedding was reached between 2 and 3 dpi in all routes of inoculation groups infected with either virus. The peak of cloacal (CL) viral excretion was reached between 2 and 3 dpi in all routes of inoculation groups infected with H3N8 LPAIV and in the IO-, IC-, and II-inoculated groups infected with H5N2 LPAIV, with a delayed and shorter peak for the IN- and IT-inoculated groups. The birds inoculated via the II route had more productive OP and CL viral shedding after infection with either LPAIV, as evidenced by higher number of swabs testing positive over the study period. In conclusion, mallards can be infected with LPAIV by various routes of inoculation, and this corroborates their high susceptibility to infection by these viruses.

Franca, M., D.E. Stallknecht, and E.W. Howerth. 2013. Expression and distribution of sialic acid influenza virus receptors in wild birds. *Avian Pathology* 42(1): 60-71.

ABSTRACT: Avian influenza (AI) viruses have been detected in more than 105 wild bird species from 12 different orders but species-related differences in susceptibility to AI viruses exist. Expression of α2,3-linked (avian-type) and α2,6-linked (human-type) sialic acid (SA) influenza virus receptors in tissues is considered one of the determinants of the host range and tissue tropism of influenza viruses. We investigated the expression of these SA receptors in 37 wild bird species from 11 different orders by lectin histochemistry. Two isoforms of Maackia amurensis (MAA) lectin, MAA1 and MAA2, were used to detect α2,3-linked SA, and *Sambucus nigra* lectin was used to detect α2,6-linked SA. All species evaluated expressed α2,3-linked and α2,6-linked SA receptors in endothelial cells and renal tubular epithelial cells. Both α2,3-linked and α2,6-linked SA receptors were expressed in respiratory and intestinal tract tissues of aquatic and terrestrial wild bird species from different taxa, but differences in SA expression and in the predominant isoform of MAA lectin bound were observed. With a few possible exceptions, these observed differences were not generally predictive of reported species susceptibility to AI viruses based on published experimental and field data.

Franca, M., D.E. Stallknecht, R. Poulson, J. Brown, and E.W. Howerth. 2012. The pathogenesis of low pathogenic avian influenza in mallards. *Avian Diseases* 56(4s1): 976-980.

ABSTRACT: Mallards are important natural hosts involved in the epidemiology of low pathogenic avian influenza viruses (LPAIVs). LPAIVs are mainly transmitted by a fecal-oral route and are excreted in high concentrations in the feces. We investigated the pathology, viral antigen distribution, and the expression of alpha2,3 sialic acid (SA) influenza virus receptors in mallards after intranasal inoculation with A/Mallard/MN/199106/99 (H3N8) or A/Mallard/MN/355779/00 (H5N2). Gross lesions were not observed. Avian influenza virus (AIV) nucleoprotein (NP) antigen was detected in rare epithelial cells of the larynx and trachea only at 1-day postinoculation (dpi) in the birds infected with H3N8 LPAI, but infection with either virus was associated with lymphocytic tracheitis and laryngitis on 1 and 2 dpi. AIV NP antigen was detected in enterocytes of the lower intestine from 1 to 4 dpi and in epithelial cells of the bursa of Fabricius from 2 to 3 dpi in birds infected with either virus. Oropharyngeal and cloacal viral shedding was detected from 1 dpi, with higher cloacal viral shedding detected at 2 and 3 dpi with both viruses. Mallards abundantly expressed alpha2,3 sialic acid receptors in epithelial cells of the respiratory tract, lower intestine, and bursa of Fabricius. Some infected birds had decreased alpha2,3 sialic acid expression in epithelial cells of the bursa of Fabricius and in enterocytes of the ceca and colon. In conclusion, the main sites of LPAIV replication in mallards are the enterocytes of the lower intestinal tract and epithelial cells of the bursa of Fabricius in the first days after infection, when these birds are shedding AIV in high titers in the feces.

Gleim, E.R., L.M. Conner, and M.J. Yabsley. 2013. The effects of *Solenopsis invicta* (Hymenoptera: Formicidae) and burned habitat on the survival of *Amblyomma americanum* (Acari: Ixodidae) and *Amblyomma maculatum* (Acari: Ixodidae). *Journal of Medical Entomology* 50(2): 270-276.

ABSTRACT: Identifying ways in which humans can reduce tick populations is important for preventing the spread and emergence of diseases. During a recent study on effects of long-term prescribed burning on ticks, differences in species composition were observed with lone star ticks, *Amblyomma americanum* (L.), preferring unburned habitats and Gulf Coast ticks, *Amblyomma maculatum* (Koch), preferring burned habitats. Interestingly, the red imported fire ant, *Solenopsis invicta* Buren, is found predominantly in disturbed habitats, such as burned habitats, and studies have reported that red imported fire ants prey on lone star ticks. To better understand drivers of tick population differences in burned habitats, the current study was conducted to evaluate the effects of red imported fire ants and habitat on survival of lone star and Gulf Coast ticks. Within treatments (burned habitat with red imported fire ants, burned habitat without red imported fire ants, and unburned habitat without red imported fire ants), 10 tick enclosures were installed and seeded with engorged lone star or Gulf Coast tick nymphs. After molting, ticks within enclosures were collected. Survival of lone star ticks in burned habitats (regardless of red imported fire ant presence) was significantly lower compared with unburned habitat. Gulf Coast ticks had significantly greater survival in burned habitats (regardless of red imported fire ant presence) compared with lone star ticks. In this study, burning status was more important for survival of ticks than presence of red imported fire ants, with Gulf Coast ticks surviving better in burned habitat that typically experiences higher temperatures and lower humidity.

Haman, K.H., T.M. Norton, R.A. Ronconi, N.M. Nemeth, A.C. Thomas, S.J. Courchesne, A. Segars, and M.K. Keel. 2013. Great shearwater (*Puffinus gravis*) mortality events along the eastern coast of the United States. *Journal of Wildlife Diseases* 49(2): 235-245.

ABSTRACT: The great shearwater (*Puffinus gravis*) is an abundant pelagic seabird that undertakes transequatorial migrations between the North and South Atlantic Ocean. This species is a useful indicator of large-scale alterations in marine dynamics due to its wide geographic range, long-distance migrations, and relative abundance. From 1993 to 2011, 12 separate mortality events, with 4,961 great shearwaters recovered, were documented along the eastern coast of the United States. Of these, seven events (n=4,885) occurred in the Southeast (SE) and five (n=76) in the Northeast (NE) United States. The cause of death was determined either by necropsy (n=60) or external examination (n=4,901). All great shearwaters stranded along the SE United States were emaciated while 58% were emaciated in the NE United States. No plastic was observed in great shearwaters in the SE US (n=27), but the gastrointestinal tract of 82% (n=27) of all stranded birds along the NE United States had at least one plastic bead. There was no evidence of infectious disease or heavy metals in stranded great shearwaters examined (n=14, from the 2005 SE event). Stable isotope analysis of feathers (n=9, from a 2007 SE event) suggests dietary differences between emaciated stranded birds and live-caught healthy birds. The temporal distribution of stranding detections suggests a general increase in the number of observed great shearwater strandings over the past two decades. From 1993 to 2000, there were a total of three mortality events with 296 individual great shearwaters. However, there was a threefold increase in the number of mortality events from 2001 to 2011 (nine events involving 4,665 individuals). The causes of this apparent increase in strandings are unknown but may be due to an increase in reporting effort over the past two decades combined with changing oceanographic conditions in the South Atlantic Ocean, leading to large-scale mortality of emaciated great shearwaters along the east coast of the United States.

Handel, A., J. Brown, D. Stallknecht, and P. Rohani. 2013. A multi-scale analysis of influenza A virus fitness trade-offs due to temperature-dependent virus persistence. *PLoS Computational Biology* 9(3): e1002989.

ABSTRACT: Successful replication within an infected host and successful transmission between hosts are key to the continued spread of most pathogens. Competing selection pressures exerted at these different scales can lead to evolutionary trade-offs between the determinants of fitness within and between hosts. Here, we examine such a trade-off in the context of influenza A viruses and the differential pressures exerted by temperature-dependent virus persistence. For a panel of avian influenza A virus strains, we find evidence for a trade-off between the persistence at high versus low temperatures. Combining a within-host model of influenza infection dynamics with a between-host transmission model, we study how such a trade-off affects virus fitness on the host population level. We show that conclusions regarding overall fitness are affected by the type of link assumed between the within- and between-host levels and the main route of transmission (direct or environmental). The relative importance of virulence and immune response mediated virus clearance are also found to influence the fitness impacts of virus persistence at low virus high temperatures. Based on our results, we predict that if transmission occurs mainly directly and scales linearly with virus load, and virulence or immune responses are negligible, the evolutionary pressure for influenza viruses to evolve toward good persistence at high within-host temperatures dominates. For all other scenarios, influenza viruses with good environmental persistence at low temperatures seem to be favored.

Hernandez, S.M., B. Galbreath, D.F. Riddle, A.P. Moore, M.B. Palamar, M.G. Levy, C.S. DePerno, M.T. Correa, and M.J. Yabsley. 2013. *Baylisascaris procyonis* in raccoons

(*Procyon lotor*) from North Carolina and current status of the parasite in the USA. *Parasitology Research* 112(2): 693-698.

ABSTRACT: *Baylisascaris procyonis* is an intestinal nematode of raccoons (*Procyon lotor*) that can cause fatal larva migrans in numerous species of birds and mammals, including humans. Historically, this parasite has been rare in the southeastern USA but recently has been reported in eastern Tennessee and isolated parts of Georgia and Florida. The objective of the current study was to investigate the distribution and prevalence of *B. procyonis* in raccoons from North Carolina. In western North Carolina, in counties bordering Tennessee, *B. procyonis* was detected in 9 of 74 (12%) raccoons sampled in 2010-2011. In general, worm burdens (average 20 worms) were low, but one raccoon had 13 adult worms. No difference was noted in prevalence by year or age, but significantly more males were infected compared with females. Sequences of the internal transcribed spacer 2 region from three samples were identical to *B. procyonis*. In central North Carolina (Guilford County), all 34 raccoons and 49 fecal samples tested were negative. Collation of data from previous studies conducted in the Southeast indicates that *B. procyonis* has been reported from numerous counties, but surveillance has been patchy and many negative results are >30 years old. These results indicate that *B. procyonis* is established in North Carolina and given the zoonotic and wildlife health implications of this parasite, additional surveillance in North Carolina and other southeastern states is warranted.

Hernandez, S.M., M.K. Keel, S. Sanchez, E. Trees, P. Gerner-Smidt, J.K. Adams, Y. Cheng, A. Ray, G. Martin, A. Presotto, M.G. Ruder, J.D. Brown, D.S. Blehert, W. Cottrell, and J.J. Maurer. 2012. Epidemiology of *Salmonella enterica* spp. *enterica* Serovar Typhimurium strain associated with a songbird outbreak. *Applied and Environmental Microbiology* 78(20): 7290-7298.

ABSTRACT: *Salmonella enterica* subsp. *enterica* serovar Typhimurium is responsible for the majority of salmonellosis cases worldwide. This *Salmonella* serovar is also responsible for die-offs in songbird populations. In 2009, there was an *S.* Typhimurium epizootic reported in pine siskins in the eastern United States. At the time, there was also a human outbreak with this serovar that was associated with contaminated peanuts. As peanuts are also used in wild-bird food, it was hypothesized that the pine siskin epizootic was related to this human outbreak. A comparison of songbird and human *S.* Typhimurium pulsed-field gel electrophoresis (PFGE) patterns revealed that the epizootic was attributed not to the peanut-associated strain but, rather, to a songbird strain first characterized from an American goldfinch in 1998. This same *S.* Typhimurium strain (PFGE type A3) was also identified in the PulseNet USA database, accounting for 137 of 77,941 total *S.* Typhimurium PFGE entries. A second molecular typing method, multiple – locus variable – number tandem – repeat analysis (MLVA), confirmed that the same strain was responsible for the pine siskin epizootic in the eastern United States but was distinct from a genetically related strain isolated from pine siskins in Minnesota. The pine siskin A3 was first encountered in May 2008 in an American goldfinch and later in a northern cardinal at the start of the pine siskin epizootic. MLVA also confirmed the clonal nature of *S.* Typhimurium in songbirds and established that the pine siskin epizootic strain was unique to the finch family. For 2009, the distribution of PFGE type A3 in passerines and humans mirrored the highest population density of pine siskins for the East Coast.

Hernandez, S.M., V.E. Peters, P.L. Weygandt, C. Jimenez, P. Villegas, B. O'Connor, M.J. Yabsley, M. Garcia, S.M. Riblet, and C.R. Carroll. 2013. Do shade-grown coffee plantations pose a disease risk for wild birds? *Ecohealth* doi: 10.1007/S10393-013-0837-3.

ABSTRACT: Shade-grown coffee plantations are often promoted as a conservation strategy for wild birds. However, these agro-ecosystems are actively managed for food production, which may alter bird behaviors or interactions that could change bird health, compared to natural forest. To examine whether there is a difference between the health parameters of wild birds inhabiting shade-grown coffee plantations and natural forest, we evaluated birds in Costa Rica for (1) their general body condition, (2) antibodies to pathogens, (paramyxovirus and *Mycoplasma* spp.), and (3) the prevalence and diversity of endo-, ecto-, and hemoparasites. We measured exposure to *Mycoplasma* spp. and paramyxovirus because these are pathogens that could have been introduced with domestic poultry, one mechanism by which these landscapes would be detrimental to wild birds. We captured 1,561 birds representing 75 species. Although seasonal factors influenced body condition, we did not find bird general body condition to be different. A total of 556 birds of 31 species were tested for antibodies against paramyxovirus-1. Of these, five birds tested positive, four of which were from shade coffee. Out of 461 other tests for pathogens (for antibodies and nucleotide detection), none were positive. *Pterolichus obtusus*, the feather mite of chickens, was found on 15 birds representing two species and all were from shade-coffee plantations. Larvated eggs of *Syngamus trachea*, a nematode typically associated with chickens, were found in four birds captured in shade coffee and one captured in forest. For hemoparasites, a total of 1,121 blood smears from 68 bird species were examined, and only one species showed a higher prevalence of infection in shade coffee. Our results indicate that shade-coffee plantations do not pose a significant health risk to forest birds, but at least two groups of pathogens may deserve further attention: *Haemoproteus* spp. and the diversity and identity of endoparasites.

Howerth, E.W., A. Olivier, M. Franca, D.E. Stallknecht, and S. Gers. 2012. Pathobiology of highly pathogenic avian influenza virus H5N2 infection in juvenile ostriches from South Africa. *Avian Diseases* 56(4s1): 966-968.

ABSTRACT: In 2011, over 35,000 ostriches were slaughtered in the Oudtshoorn district of the West Cape Province of South Africa following the diagnosis of highly pathogenic avian influenza virus H5N2. We describe the pathology and virus distribution via immunohistochemistry in juvenile birds that died rapidly in this outbreak after showing signs of depression and weakness. Associated sialic acid (SA) receptor distribution in uninfected birds is also described. At necropsy, enlarged spleens, swollen livers, and generalized congestion were noted. Birds not succumbing to acute influenza infection often became cachectic with serous atrophy of fat, airsacculitis, and secondary infections. Necrotizing hepatitis, splenitis, and airsacculitis were prominent histopathologic findings. Virus was detected via immunohistochemistry in abundance in the liver and spleen but also in the air sac and gastrointestinal tract. Infected cells included epithelium, endothelium, macrophages, circulating leukocytes, and smooth muscle of a variety of organs and vessel walls. Analysis of SA receptor distribution in uninfected juvenile ostriches via lectin binding showed abundant expression of SAalpha2,3Gal (avian type) and little or no expression of SAalpha2,6Gal (human type) in the gastrointestinal and respiratory tracts, as well as leukocytes in the spleen and endothelial cells in all organs, which correlated with H5N2 antigen distribution in these tissues.

Ishtiaq, F., M. Gilbert, J.D. Brown, P. Joyner, R. Sodnomdarjaa, M. P. Luttrell, D.E. Stallknecht, and D.O. Joly. 2012. Antibodies to influenza A virus in wild birds across Mongolia, 2006-2009. *Journal of Wildlife Diseases* 48(3): 768-775.

ABSTRACT: Wild waterbirds sampled July 2006-September 2009 in Mongolia were tested for antibodies to avian influenza (AI) virus with the use of a commercially available blocking

enzyme-linked immunosorbent assay. Antibodies were detected in 25% (572/2,282) of tested birds representing 26 species, and all antibody-positive samples were from 12 species in the orders Anseriformes and Charadriiformes. The highest antibody prevalence was in ruddy shelducks (*Tadorna ferruginea*; 61.7%; n=261; 95% confidence interval [CI] 55.8-67.6%), whooper swans (*Cygnus cygnus*; 38.4%; n=242; 95% CI 32.3-44.5%), swan geese (*Anser cygnoides*; 15%; n=127; 95% CI 8.6-21.4%), bar-headed geese (*Anser indicus*; 13%; n=738; 95% CI 10.3-15.1%), and Mongolian gulls (*Larus mongolicus*; 3.9%; n=255; 95% CI 1.3-6.5%). There was no significant temporal or spatial variation in the presence of antibodies in the sampled species. However, bar-headed geese and Mongolian gulls showed spatial variation in antibody prevalence in 2007 and 2008, respectively. Our study provides insights into the hatch year waterbirds' exposure to AI virus at their natal and molting sites in Mongolia.

Keel, M.K., D.E. Stallknecht, D. Cobb, M. Cunningham, V. Goekjian, S. Gordon-Akhvlediani, and J.R. Fischer. 2013. The epizootiology of anatid herpesvirus 1 infection in free-flying waterfowl: a comparison of latent and active infections among native waterfowl, captive-reared released ducks and peridomestic or feral ducks. *Journal of Wildlife Diseases* 49(3): 486-491.

ABSTRACT: The epizootiology of anatid herpesvirus 1 (AHV-1) infection in waterfowl is poorly understood but apparently involves persistence of the virus in latently infected birds. Eporniticis have often occurred in captive waterfowl or semiwild ducks in parklike settings, and many wildlife professionals conclude that such ducks may be the source of infection for wild waterfowl. We assessed the prevalence of latent infection and viral shedding from four groups of waterfowl: naturally occurring populations of native waterfowl, captive-reared waterfowl released for shooting, introduced nonmigratory waterfowl (e.g., resident, wild mallards; *Anas platyrhynchos*), and semiwild peridomestic waterfowl (e.g., park ducks) in North Carolina and Florida, USA, from 2004 to 2009. A nested PCR assay was used to detect viral DNA in trigeminal ganglia and cloacal swabs. Detection of viral DNA in trigeminal ganglia, but not cloacal swabs, was assumed to indicate latent infection, whereas PCR-positive cloacal swabs indicated active shedding of the virus. We collected 2,045 samples from 23 species of native, wild waterfowl, and detected latent infections in nine species. Wild northern pintails (*Anas acuta*), a species reportedly resistant to the virus, had the highest prevalence (8.1%). However, low prevalences were identified in other waterfowl from various families. Cloacal shedding was rarely detected (0.1% prevalence) among native waterfowl and was observed in one blue-winged teal (*Anas discors*) and one mottled duck (*Anas fulvigula*). All captive-reared, released waterfowl (n=13) collected were mallards and one was latently infected, suggesting that these birds could also serve as a source of AHV-1 for naïve waterfowl. All nonmigratory waterfowl sampled (n=90) were also mallards. None of the resident mallards were shedding virus, but one was latently infected. The peridomestic waterfowl sampled included breeds of domestic mallard (n=6) and Muscovy ducks (*Cairina moschata*; n=73). One peridomestic mallard and four Muscovy ducks were shedding virus at the time they were sampled, but no latently infected, asymptomatic carriers were identified.

Keeler, S.P., R.D. Berghaus, and D.E. Stallknecht. 2012. Persistence of low pathogenic avian influenza viruses in filtered surface water from waterfowl habitats in Georgia, USA. *Journal of Wildlife Diseases* 48(4): 999-1009.

ABSTRACT: The natural reservoirs for avian influenza virus (AIV) are wild bird species of the orders Anesiformes and Charadiiformes. The primary route of transmission for wild birds is through fecally contaminated surface water on shared aquatic habitats. A distilled water

model has shown that AIV remains infectious in water for weeks to months with pH, salinity, and temperature affecting stability. To evaluate the effect of pH, salinity, and temperature on AIV persistence in natural surface water, we measured the duration of infectivity for two common low pathogenic AIV subtypes in 15 filtered surface water samples collected from major waterfowl habitats in Georgia, USA. Trials were performed at three incubation temperatures 10, 17, and 28 C. Consistent with previous studies, pH and temperature had a significant effect on the stability of AIV in filtered surface water. Both viruses were less stable at warmer temperatures and in acidic water (pH <5.0). Due to the limited range of salinity of the field water samples, the role of salinity in AIV stability in surface water could not adequately be evaluated. Variations in persistence times between water samples with comparable pH and salinities indicated that other factors affect AIV stability in natural surface water. These results contribute to the current understanding of AIV persistence in aquatic habitats and may help in identifying areas with an increased likelihood of AIV persistence and potential transmission.

Keeler, S.P., C. Lebarbenchon, and D.E. Stallknecht. 2013. Strain-related variation in the persistence of influenza A virus in three types of water: distilled water, filtered surface water, and intact surface water. *Virology Journal* 10(1): 13.

ABSTRACT: The persistence of influenza A (AI) virus in aquatic habitats has been demonstrated to be a determinant for virus transmission dynamics in wild duck populations. In this study, we investigated virus strain-related variation in persistence in water for nine wild duck isolated IA viruses of three subtypes (H3N8, H4N6, and H8N4). We experimentally estimated the loss of infectivity over time in three different types of water: distilled, filtered surface water, and intact surface water. All viruses persisted longest in distilled water followed by filtered surface water with markedly reduced durations of persistence observed in the intact surface water. Strain-related variations were observed in distilled and filtered surface water but limited variation was observed in the intact surface water. Our findings suggest that the role of surface water for long-term (between years) maintenance of AI viruses in the environment may be limited, and suggest that the physicochemical characteristics of water, as well as microorganisms, may be of strong importance. Results also indicate that the extent of strain-related variation observed in distilled water may overestimate persistence abilities for IA viruses in the wild and supports the need to develop experiments that account for these effects to assess subtype, genotype, as well as spatial and temporal variation in the persistence of IA viruses in aquatic habitats.

Keeler, S.P., M.J. Yabsley, J.M. Fox, S.N. McGraw, and S.M. Hernandez. 2012. *Isospora troglodytes* n. sp. (Apicomplexa: Eimeriidae), a new coccidian species from wrens of Costa Rica. *Parasitology Research* 110(5): 1723-1725.

ABSTRACT: Nineteen (91%) of 21 rufous-and-white wrens (*Thryothorus rufalbus*) and five (71%) of seven plain wrens (*Cantorchilus modestus*) sampled from Costa Rica were positive for a new species of *Isospora*. Oocysts have a thin, smooth, double, colorless wall and measure 20.1 ± 1.4 x 23.4 ± 1.5 µm (18-24 x 20-26 µm) with an average length-width ratio of 1.2 µm. Sporocysts are ovoid, measure 9.5 ± 0.9 x 15.5 ± 1.1 µm (7-2 x 12-18 µm) with an average length-width ratio of 1.6 µm. A nipple-like steida body continuous with the sporocyst wall and a prominent oval-shaped substeida body are present. In addition to the four sporozoites, a single compact sporocyst residuum was present in each sporocyst. This is the first description of an *Isospora* species from the family Troglodytidae and the first report of *Isospora* from the rufous-and-white wren and plain wren.

Kistler, W.M., S.M. Hernandez, S.E. Gibbs, J.R. Ballard, S.L. A.T. Johnson, and M.J. Yabsley. 2013. Evaluation of a restriction fragment length enzyme assay for differentiation of *Haemoproteus* and *Plasmodium* across a standard region of the Mitochondrial genome. *Journal of Parasitology* doi: 10.1645/13-211.1.

ABSTRACT: Avian haemosporidian parasites are a genetically diverse group of parasites with a near cosmopolitan distribution. Over the past two decades, several PCR protocols have been designed to detect these parasites. The majority of these protocols amplify part of, or the entire mitochondrial cytochrome b gene. However, many of these protocols co-amplify two genera (*Haemoproteus* and *Plasmodium*) making it impossible to determine which genus is amplified without post-PCR analysis. A uniform database (MalAvi), containing sequences amplified with the primers HAEMF and HAEMR2, has been developed to increase comparability across studies. We analyzed sequences from the MalAvi database and new sequences and found that digestion with EcoRV could be used to distinguish *Haemoproteus* from the majority of *Plasmodium* sequences. In addition, we tested 220 wild birds from Costa Rica and the United States for avian haemosporidians and assessed the ability of EcoRV to distinguish these two genera. Thirty-six positive samples were sequenced to confirm the restriction profiles, and we also analyzed 63 new haemosporidian sequences from ongoing studies in the United States for the restriction site. Among these new samples, all of the 85 *Haemoproteus* (subgenus *Parahaemoproteus*) and 14 *Plasmodium* were distinguishable. Overall, 887 of 898 (98.8%) sequences from our studies and the MalAvi database were assigned to the correct genus. Of these samples, all *Haemoproteus* samples were correctly identified, and all but 11 *Plasmodium* samples were correctly identified by the EcoRV assay. Overall, this restriction enzyme protocol is able to quickly and efficiently classify these two genera of avian malarial parasites and would be useful for researchers interested in identifying parasites to genus-level, studies focused on sequence analysis of only a single genus, or for detecting co-infections that would need cloning prior to sequence analysis.

Kistler, W.M., D.E. Stallknecht, T.J. Deliberto, S. Swafford, K. Pedersen, K. Van Why, P.C. Wolf, J.A. Hill, D.L. Bruning, J.C. Cumbee, R.M. Mickley, C.W. Betsill, A.R. Randall, R.D. Berghaus, and M.J. Yabsley. 2012. Antibodies to avian influenza viruses in Canada geese (*Branta canadensis*): a potential surveillance tool. *Journal of Wildlife Diseases* 48(4): 1097-1101.

ABSTRACT: Traditionally, the epidemiology of avian influenza viruses (AIVs) in wild birds has been defined by detection of virus or viral RNA through virus isolation or reverse-transcription polymerase chain reaction. Our goals were to estimate AIV antibody prevalence in Canada geese (*Branta canadensis*) and measure effects of age and location of these estimates. We collected 3,205 samples from nine states during June and July 2008 and 2009: Georgia, Massachusetts, Minnesota, Mississippi, New Jersey, North Carolina, Pennsylvania, Washington, and West Virginia. Serum samples were tested for AIV antibodies with the use of a commercial blocking enzyme-linked immunosorbent assay. Overall, 483 (15%) Canada geese had detectable antibodies to AIV. Significantly higher prevalences were detected in geese collected from northeastern and upper midwestern states compared with southeastern states. This trend is consistent with results from virus isolation studies reporting AIV prevalence in North American dabbling ducks. Within Pennsylvania, significantly higher antibody prevalences were detected in goose flocks sampled in urban locations compared to flocks sampled in rural areas. Antibody prevalence was significantly higher in after-hatch-year geese compared to hatch-year geese. No significant differences in prevalence were detected from 10 locations sampled during both years. Results indicate that Canada geese are frequently exposed to AIVs and, with

resident populations, may potentially be useful as sentinels to confirm regional AIV transmission with wild bird populations.

Kocer, Z.A., S. Krauss, D.E. Stallknecht, J.E. Rehg, and R.G. Webster. 2012. The potential for avian H1N1 influenza A viruses to replicate and cause disease in mammalian models. *PLoS One* 7(7): e41609.

ABSTRACT: H1N1 viruses in which all gene segments are of avian origin are the most frequent cause of influenza pandemics in humans; therefore, we examined the disease-causing potential of 31 avian H1N1 isolates of American lineage in DBA/2J mice. Thirty of 31 isolates were very virulent, causing respiratory tract infection; 22 of 31 resulted in fecal shedding; and 10 of 31 were as pathogenic as the pandemic 2009 H1N1 viruses. Preliminary studies in BALB/cJ mice and ferrets showed that 1 of 4 isolates tested was more pathogenic than the pandemic 2009 H1N1 viruses in BALB/cJ mice, and 1 of 2 strains transmitted both direct and respiratory-droplet contact in ferrets. Preliminary studies of other avian subtypes (H2, H3, H4, H6, H10, H12) in DBA/2J mice showed lower pathogenicity than the avian H1N1 viruses. These findings suggest that avian H1N1 influenza viruses are unique among influenza A viruses in their potential to infect mammals.

LaDouceur, E.E., J. Ernst, and M.K. Keel. 2012. Unilateral corneoscleral choristomas (corneal dermoids) in a white-tailed deer (*Odocoileus virginianus*). *Journal of Wildlife Diseases* 48(3): 826-828.

ABSTRACT: Multiple, nodular, pigmented masses protruding from the cornea and adjacent sclera of the left eye of a white-tailed deer (*Odocoileus virginianus*) were diagnosed as choristomas (dermoids). Microscopically, the masses contained well-differentiated skin, cartilage, and bone. This appears to be the first report of a coroneoscleral choristoma in a cervid.

Laing, S.T., E.S. Weber, M.J. Yabsley, B.C. Shock, C. Grosset, O.A. Petritz, B. Barr, C.M. Reilly, and L.J. Lowenstine. 2013. Fatal hepatic tetratrichomoniasis in a juvenile Waldrapp ibis (*Geronticus eremita*). *Journal of Veterinary Diagnostic Investigation* 25(2): 277-281.

ABSTRACT: Waldrapp ibis (*Geronticus eremita*) are a critically endangered species, and there are currently more birds in captivity than in the wild. A juvenile, male Waldrapp ibis housed in a mixed-species exhibit was found dead with no premonitory signs. Necropsy revealed extensive necrotizing hepatitis associated with numerous pleomorphic protozoa that were immunohistochemically reactive with antibodies raised against *Tritrichomonas foetus*, a parasite of cattle. Electron microscopy confirmed the organisms as members of family Trichomonadidae, and sequence analysis of the first ribosomal internal transcribed spacer region (ITS1), 5.8S ribosomal RNA, and ITS2 regions indicated high genetic similarity (96-97%) to members of the *Tetratrichomonas gallinarum* complex. The animal was born in captivity, and no introductions in this exhibit had occurred since 2009. Other Waldrapp ibis that had contact with the infected male were negative for flagellate infections by fecal examination, thus cross-species transmission is proposed as the source of infection. The host range of the *T. gallinarum* complex is very large and although the pathogenicity of its members, especially for wild birds, is controversial, these parasites should be considered as a possible cause of acute mortality in Waldrapp ibis. In addition, immunohistochemistry with *T. foetus* antibodies and molecular diagnostics may be useful tools for preventative veterinary care of endangered bird populations. A greater understanding of the ecology and pathogenesis of this pathogen may also be vital for

screening subclinical captive populations and existing wild populations prior to reintroduction efforts.

Lebarbenchon, C., S. Sreevatsan, T. Lefevre, M. Yang, M.A. Ramakrishnan, J.D. Brown, and D.E. Stallknecht. 2012. Reassortment of influenza A viruses in wild duck populations: effects on viral shedding and persistence in water. *Proceedings of Royal Society B* 279(1744): 3967-3975.

ABSTRACT: Wild ducks of the genus *Anas* represent the natural hosts for a large genetic diversity of influenza A viruses. In these hosts, co-infections were different virus genotypes are frequent and result in high rates of genetic reassortment. Recent genomic data have provided information regarding the pattern and frequency of these reassortant viruses in duck populations; however, potential consequences on viral shedding and maintenance in the environment have not been investigated. On the basis of full genome sequencing, we identified five virus genotypes, in a wild duck population in northwestern Minnesota (USA) that naturally arose from genetic reassortments. We investigated the effects of influenza A virus genotype on the viral shedding pattern in mallards (*Anas platyrhynchos*) and the duration of infectivity in water, under different temperature regimens. Overall, we found that variation in the viral genome composition of these isolates had limited effects on duration, extent and pattern of viral shedding, as well as on the reduction of infectivity in water over time. These results support that, in wild ducks, functionally equivalent gene segments could be maintained in virus populations with no fitness costs when genetic reassortments occur.

Leiser, O.P., J.L. Corn, B.S. Schmit, P.S. Keim, and J.T. Foster. 2013. Feral swine brucellosis in the United States and prospective genomic techniques for disease epidemiology. *Veterinary Microbiology* 166(1-2): 1-10.

ABSTRACT: Brucellosis is a common infection of feral swine throughout the United States. With the recent expansion of feral swine populations across the country, this disease poses an increasing threat to agriculture and hunters. The standard approach to *Brucella* surveillance in feral swine has been serological testing, which gives an indication of past exposure and is a rapid method of determining populations where *Brucella* is present. More in-depth analyses require bacterial isolation to determine the *Brucella* species and biovar involved. Ultimately, for a comprehensive understanding of *Brucella* epizootiology in feral swine, incorporation of genotyping assays has become essential. Fortunately, the past decade has given rise to an array of genetic tools for assessing *Brucella* transmission and dispersal. This review aims to synthesize what is known about brucellosis in feral swine and will cover prospective genomic techniques that may be utilized to develop more complete understanding of the disease and its transmission history.

Macaluso, K., C.D. Paddock, and M.J. Yabsley. 2013. Pathogen Biology and Ecology. In: *Proceedings of a Regional Workshop to Assess Research and Outreach Needs in Integrated Pest Management to Reduce the Incidence of Tick-borne Diseases in the Southern United States,* pp. 31-42. C.S. Anderson and W.L. Nicholson (Editors). Centers for Disease Control and Prevention, Atlanta, Georgia.

Maxted, A.M., R.R. Porter, M.P. Luttrell, V.H. Goekjian, A.D. Dey, K.S. Kalasz, L.J. Niles, and D.E. Stallknecht. 2012. Annual survival of ruddy turnstones is not affected by natural infection with low pathogenicity avian influenza viruses. *Avian Diseases* 56(3): 567-573.

ABSTRACT: The population of ruddy turnstones (*Arenaria interpres morinella*) that migrates through Delaware Bay has undergone severe declines in recent years, attributable to

reduced availability of horseshoe crab (*Limulus polyphemus*) eggs at this critical spring migration stopover site. Concurrently, this population has experienced annual low pathogenicity avian influenza virus (AIV) epidemics at this same site. Using a prospective cohort study design with birds individually flagged during May-June 2006-2008, we evaluated resighting rates (a proxy for annual survival) between AIV-infected and uninfected birds at one year after capture, testing, and measurement. Overall resighting rate was 46%, which varied by year and increased with relative mass of the bird when captured. Resighting rates were not different between AIV-infected and uninfected birds in any period. In multivariate analyses, infection status was also unrelated to resighting rate after controlling for year, day, state, sex, body size, mass index, or whether the bird was blood-sampled. Thus, apparent annual survival in ruddy turnstones was not reduced by AIV infection at this migratory stopover. However, it is unknown whether intestinal AIV infection might cause subtle reductions in weight gain which could negatively influence reproduction.

Mertins, J.W., and J.L. Corn. 2013. LabNotes: Equine piroplasmosis and wildlife ectoparasites in the United States, especially South Texas. *VMO Observer,* Animal and Plant Health Inspection Service, United States Department of Agriculture.

Moulis, R.A., H.B. Lewandowski, J.D. Russell, J.L. Heusel, L.F. Peaty, D.G. Mead, and R. Kelly. 2013. West Nile virus activity in Chatham County, Georgia, during 2011. *Wing Beats Spring:* 23-27.

Nemeth, N.M., J.D. Brown, D.E. Stallknecht, E.W. Howerth, S.H. Newman, and D.E. Swayne. 2013. Experimental infection of bar-headed geese (*Anser indicus*) and ruddy shelducks (*Tadorna ferruginea*) with a clade 2.3.2 H5N1 highly pathogenic avian influenza virus. *Veterinary Pathology* doi: 10.1177/0300985813490758.

ABSTRACT: Since 2005, clade 2.2 H5N1 highly pathogenic avian influenza (HPAI) viruses have caused infections and morbidity among numerous species of wild waterfowl in Eurasia and Africa. However, outbreaks associated with clade 2.3.2 viruses have increased since 2009, and viruses within this clade have become the dominant strain of the H5N1 HPAI virus detected in wild birds, reaching endemic status in domestic birds in selected regions of Asia. To address questions regarding the emergence and expansion of clade 2.3.2 viruses, two waterfowl species repeatedly involved in outbreaks of H5N1 HPAI viruses, bar-headed geese (*Anser indicus*) and ruddy shelducks (*Tadorna ferruginea*), were inoculated with a representative virus. All of three infected ruddy shelducks exhibited neurologic signs and died within four to five days. Two of three infected bar-headed geese had transient weakness but all survived. Viral shedding was predominately via the oropharynx and was detected from 1 to 7 days after inoculation. The severity and distribution of microscopic lesions corresponded with clinical disease and influenza-specific immunohistochemical staining of neurons. The predominant lesions were in the brain and were more severe in ruddy shelducks. Increased caspase-3 reactivity in the brains of all infected birds suggests a role for apoptosis in H5N1 HPAI virus pathogenesis in these species. These results demonstrate that similar to clade 2.2 viruses, a clade 2.3.2 H5N1 HPAI virus is neurotropic in some waterfowl species and can lead to neurologic disease with varying clinical outcomes. This has implications for the role that wild waterfowl may play in transmission of this virus in endemic regions.

Nemeth, N.M., P.T. Oesterle, R.L. Poulson, C.A. Jones, S.M. Tomkins, J.D. Brown, and D.E. Stallknecht. 2013. Experimental infection of European starlings (*Sturnus vulgaris*) and house sparrows (*Passer domesticus*) with pandemic 2009 H1N1 and swine H1N1 and H3N2 triple reassortant influenza viruses. *Journal of Wildlife Diseases* 49(2): 437-440.

ABSTRACT: European starlings (*Sturnus vulgaris*) and house sparrows (*Passer domesticus*) are common peridomestic passerine birds that are often associated with domestic animal production facilities. This association provides a potential means for pathogen transmission between facilities. We inoculated European starlings and house sparrows with three non-avian influenza virus strains: two swine isolates (H1N1 and H3N2) and one human isolate representing the H1N1 pandemic strain that originated from swine. No viral shedding was observed in house sparrows, and shedding was minimal and transient in two of 12 (17%) European starlings. One of these two infected starlings seroconverted 14 days after inoculation. These results suggest that these two passerine species are minimally susceptible to current influenza viruses in domestic pigs and therefore pose a negligible risk for transmission between or within swine production facilities.

Noel, B.L., J.C. Bednarz, M.G. Ruder, and M.K. Keel. 2013. Effects of radio-transmitter methods on pileated woodpeckers: an improved technique for large woodpeckers. *Southeastern Naturalist* 12(2): 399-412.

ABSTRACT: We captured and radio-marked 64 *Dryocopus pileatus* (pileated woodpecker) in bottomland hardwood forests from February 2007 to June 2010. At least 12 (35.3%) of the first 34 birds radio-tagged died within 43 d of capture (x = 8.2 d). Thus, we adjusted our radio-attachment techniques adaptively from a figure-eight harness to a tail-mount, and reduced handling times to minimize stress on woodpeckers. In 2009 and 2010, after the change in attachment type and modified handling protocol including a reduction of handling time (from ca. 1 h to 30 min), all 30 radio-marked birds (100%) survived the entire field season (≥3 mo). These data suggested that pileated woodpeckers, and perhaps other large woodpeckers, have an increased risk of death when tagged with figure eight harnesses, handled for longer periods and more obtrusively, and captured on days with relatively cold temperatures. We submit that future telemetry on this species or other large woodpeckers should not employ the figure-eight harnesses and should strive to minimize handling time and disturbance. We recommend that other ornithologists observing higher than expected mortalities possibly related to handling birds or transmitter attachment publish this information to minimize the adverse impacts of birds during future research.

Park, A.W., K. Magori, B.A. White, and D.E. Stallknecht. 2013. When more transmission equals less disease: reconciling the disconnect between disease hotspots and parasite transmission. *PLoS One* 8(4): e61501.

ABSTRACT: The assumed straightforward connection between transmission intensity and disease occurrence impacts surveillance and control efforts along with statistical methodology, including parameter inference and niche modeling. Many infectious disease systems have the potential for this connection to be more complicated-although demonstrating this in any given disease system has remained elusive. Hemorrhagic disease (HD) is one of the most important diseases of white-tailed deer and is caused by viruses in the *Orbivirus* genus. Like many infectious diseases, the probability or severity of disease increases with age (after loss of maternal antibodies) and the probability of disease is lower upon re-infection compared to first infection (based on cross-immunity between virus strains). These broad criteria generate a prediction that disease occurrence is maximized at intermediate levels of transmission intensity. Using published U.S. field data, we first fit a statistical model to predict disease occurrence as a function of seroprevalence (a proxy for transmission intensity), demonstrating that states with intermediate seroprevalence have the highest level of case reporting. We subsequently introduce an independently parameterized mechanistic model supporting the theory that high case reporting should come from areas with intermediate levels of transmission. This is the first

rigorous demonstration of this phenomenon and illustrates that variation in transmission rate (e.g. along an ecologically-controlled transmission gradient) can create cryptic refuges for infectious diseases.

Patel, J.M., A.C. Rosypal, K.L. Zimmerman, W.E. Monroe, N. Sriranganathan, A.M. Zajac, M.J. Yabsley, and D.S. Lindsay. 2012. Isolation, mouse pathogenicity, and genotyping of *Trypanosoma cruzi* from an English cocker spaniel from Virginia, USA. *Veterinary Parasitology* 187(304): 394-398.

ABSTRACT: *Trypanosoma cruzi* was demonstrated in blood smears and heart tissue from a five-year-old, female English cocker spaniel that had never been outside of the state of Virginia, USA. Plasma from the dog was positive in a commercially available immunochromatographic dipstick assay for *T. cruzi* and negative in an immunochromatographic dipstick assay for visceral *Leishmania* spp. The plasma from the dog had an indirect immunofluorescent antibody titer of 1:800 against epimastigoates of *T. cruzi* while the titer was 1:50 against promastigotes of *L. infantum*. The parasite was isolated from the blood in vitro from the dog (TcVT-1 isolate) and used to experimentally infect female C3H and ICR mice. The parasite was nonpathogenic for experimentally inoculated mice. DNA was isolated from parasites grown in vitro and used to determine that the genotype of *T. cruzi* present in the dog was genotype TcIV. This genotype is common in raccoons, *Procyon lotor*, in North America and suggests that raccoons may serve as reservoirs for canine infection.

Ramey, A.M., J.P. Fleskes, J.A. Schmutz, and M.J. Yabsley. 2013. Evaluation of blood and muscle tissues for molecular detection and characterization of hematozoa infections in northern pintails (*Anas acuta*) wintering in California. *International Journal for Parasitology: Parasites and Wildlife* 2(2013): 102-109.

ABSTRACT: Information on the molecular detection of hematozoa from different tissue types and multiple years would be useful to inform sample collection efforts and interpret results of meta-analyses or investigations spanning multiple seasons. In this study, we tested blood and muscle tissue collected from northern pintails (*Anas acuta*) during autumn and winter of different years to evaluate prevalence and genetic diversity of *Leucocytozoon, Haemoproteus*, and *Plasmodium* infections in this abundant waterfowl species of the Central Valley of California. We first compared results for paired blood and wing muscle samples to assess the utility of different tissue types for molecular investigations of haemosporidian parasites. Second, we explored inter-annual variability of hematozoa infection in Central Valley northern pintails and investigated possible effects of age, sex, and sub-region of sample collection on estimated parasite detection probability and prevalence. We found limited evidence for differences between tissue types in detection probability and prevalence of *Leucocytozoon, Haemoproteus*, and *Plasmodium* parasites, which supports the utility of both sample types for obtaining information on hematozoan infections. However, we detected 11 haemosporidian mtDNA cyt *b* haplotypes in blood samples vs. six in wing muscle tissue collected during the same sample year suggesting an advantage to using blood samples for investigations of genetic diversity. Estimated prevalence of *Leucocytozoon* parasites was greater during 2006-2007 as compared to 2011-2012 and four unique haemosporidian mtDNA cyt *b* haplotypes were detected in the former sample year but not in the latter. Seven of 15 mtDNA cyt *b* haplotypes detected in northern pintails had 100% identity with previously reported hematozoa lineages detected in waterfowl (*Haemoproteus* and *Leucocytozoon*) or other avian taxa (*Plasmodium*) providing support for lack of host specificity for some parasite lineages.

Reis, A., D. Stallknecht, C. Ritz, and M. Garcia. 2012. Tenacity of low pathogenic avian influenza viruses in different types of poultry litter. *Poultry Science* 91: 1745-1750.

ABSTRACT: To determine the risk of infection associated with exposure to low pathogenic avian influenza (AI) virus-contaminated poultry litter, the tenacity of low pathogenic A/Ck/CA/431/00(H6N2), A/Mallard/MN/355779/00(H5N2), and A/turkey/Ohio/313053/04 (H3N2) was evaluated. Viral stocks were incubated with poultry litter from commercial flocks at 25°C. Three types of poultry litter, wood shavings, shavings plus gypsum, and shavings plus peanut hulls, from commercial broiler flocks were used. The three low pathogenic avian influenza viruses retained infectivity for one day in wood shavings and shavings plus peanut hulls litter types, whereas in wood shavings plus gypsum, litter viruses remained infective for up to 3 d. In contrast to the survivability in litter, all the viruses maintained infectivity in water for 4 d at titers of $\log_{10}4.5$. The infectivity of A/Ck/CA/431/00(H6N2) shed by experimentally infected layers, broilers, and turkeys was retained for one day, independently of the type of litter. In commercial production where a high density of birds are housed, the viral load shed by an infected flock will be significantly higher than the viral load shed 3 d postinfection obtained under the experimental conditions used in this study. Therefore proper management and disposal of poultry by products, such as windrow composting of litter and the composting of carcasses during an AI outbreak should be implemented.

Roellig, D.M., M.Y. Savage, A.W. Fujita, C. Barnabe, M. Tibayrenc, F.J. Steurer, and M.J. Yabsley. 2013. Genetic variation and exchange in *Trypanosoma cruzi* isolates from the United States. *PLoS One* 8(2): e56198.

ABSTRACT: *Trypanosoma cruzi*, the causative agent of Chagas disease, is a multiclonal parasite with high levels of genetic diversity and broad host and geographic ranges. Molecular characterization of South American isolates of *T.* cruzi has demonstrated homologous recombination and nuclear hybridization, as well as the presence of six main genetic clusters of "discrete typing units" (DTUs). Few studies have extensively investigated such exchange events and genetic diversity in North American isolates. In the current study, we genetically characterized over 50 U.S. isolates from wildlife reservoirs (e.g., raccoons, opossums, armadillos, skunks), domestic dogs, humans, nonhuman primates, and reduviid vectors from nine states (TX, CA, OK, SC, FL, GA, MD, LA, and TN) using a multilocus sequencing method. Single nucleotide polymorphisms were identified in sequences of the mismatch-repair class 2 (MSH2) and Tc52 genes. Typing based on the two genes often paralleled genotyping by classic methodologies using mini-exon and 18S and 24Sα rRNA genes. Evidence for genetic exchange was obtained by comparing sequence phylogenies of nuclear and mitochondrial gene targets, dihydrofolate reductase-thymidylate synthase (DHFR-TS) and the cytochrome oxidase subunit II- NADH dehydrogenase subunit I region (COII-ND1), respectively. We observed genetic exchange in several U.S. isolates as demonstrated by incongruent mitochondrial and nuclear genes phylogenies, which confirms a previous finding of a single genetic exchange event in a Florida isolate. The presence of SNPs and evidence of genetic exchange illustrates that strains from the U.S. are genetically diverse, even though only two phylogenetic lineages have been identified in this region.

Rossow, J.A., S.M. Hernandez, S.M. Sumner, B.R. Altman, C.G. Crider, M.B. Gammage, K.M. Segal, and M.J. Yabsley. 2013. Haemogregarine infections of three species of aquatic freshwater turtles from two sites in Costa Rica. *International Journal for Parasitology: Parasites and Wildlife* 2: 131-135.

ABSTRACT: Twenty-five black river turtles (*Rhinoclemmys funerea*) and eight white-lipped mud turtles (*Kinosternon leucostomum*) from Selva Verde, Costa Rica, were examined for haemoparasites. Leeches identified as *Placobdella multilineata* were detected on individuals from both species. All turtles sampled were positive for intraerythrocytic haemogregarines (Apicomplex: Adeleorina) and the average parasitemia of black river turtles (0.34% ± 0.07) was significantly higher compared to white-lipped mud turtles (0.05% ± 0.006). No correlation was found between parsitemia and relative body mass of either species or between black river turtles from the two habitats. In addition, one scorpion mud turtle (*Kinosternon scorpioides*) examined from La Pacifica, Costa Rica, was positive for haemogregarines (0.01% parasitemia*).* Interestingly, parasites of the scorpion mud turtle was significantly smaller than those from the other two species and did not displace the erythrocyte nucleus, whereas parasites from the other two species consistently displaced host cell nuclei and often distorted size and shape of erythrocytes. This is the first report of haemogregarines in turtles from Central America and of haemogregarines in *K. leucostomum, K. scorpioides,* and any *Rhinoclemmys species*. Additional studies are needed to better characterize and understand the ecology of these parasites.

Rothermel, B.B., E.R. Travis, D.L. Miller, R.L. Hill, J.L. Gonynor-McGuire, and M.J. Yabsley. 2013. High occupancy of stream salamanders despite high Ranavirus prevalence in a southern Appalachians watershed. *EcoHealth* doi: 10.1007/S10393-013-0843-5.

ABSTRACT: The interactive effects of environmental stressors and emerging infectious disease pose potential threats to stream salamander communities and their headwater stream ecosystems. To begin assessing these threats, we conducted occupancy surveys and pathogen screening of stream salamanders (Family Plethodontidae) in a protected southern Appalachians watershed in Georgia and North Carolina, USA. Of the 101 salamanders screened for both chytrid fungus (*Batrachochytrium dendrobatidis*) and Ranavirus, only two exhibited low-level chytrid infections. Prevalence of Ranavirus was much higher (30.4% among five species of *Desmognathus*). Despite the ubiquity of ranaviral infections, we found high probabilities of site occupancy (≥0.60) for all stream salamander species.

Ruder, M.G., A.B. Allison, D.E. Stallknecht, D.G. Mead, S.M. McGraw, D.L. Carter, S.V. Kubiski, C.A. Batten, E. Klement, and E.W. Howerth. 2012. Susceptibility of white-tailed deer (*Odocoileus virginianus*) to experimental infection with epizootic hemorrhagic disease virus serotype 7. *Journal of Wildlife Diseases* 48(3): 676-685.

ABSTRACT: During the fall of 2006, in Israel, epizootic hemorrhagic disease virus (EHDV) serotype 7 caused an intense and widespread epizootic in domestic cattle that resulted in significant economic losses for the dairy industry. The susceptibility of potential North American vector and ruminant hosts to infection with EHDV-7 is not known but is essential to understanding the potential for establishment of this exotic orbivirus in North America if it was introduced. Our primary objective was to determine whether white-tailed deer (WTD; *Odocoileus virginianus*) are susceptible to infection with EHDV-7. Six, eight-month-old WTD were experimentally infected with EHDV-7, and all became infected and exhibited varying degrees of clinical disease. Clinical signs, clinicopathologic abnormalities, and postmortem findings were consistent with previous reports of orbiviral hemorrhagic disease (HD) in this species. Four of six animals died or were euthanized because of the severity of disease, one on postinoculation day (PID) 5 and the remaining WTD on PID 7. All deer had detectable viremia on PID 3, which peaked on PID 5 or 6 and persisted for as long as PID 46 in one animal. Deer surviving the acute phase of the disease seroconverted by PID 10. Based on the 67% mortality rate we observed, this strain of EHDV-7 is virulent in WTD,

reaffirming their role as a sentinel species for the detection of endemic and nonendemic EHDV. Further, the observed disease was indistinguishable from previous reports of disease caused by North American EHDV and bluetongue virus serotypes, highlighting the importance of serotype-specific diagnostics during suspected HD outbreaks.

Ruder, M.G., E.W. Howerth, D.E. Stallknecht, A.B. Allison, D.L. Carter, B.S. Drolet, E. Klement, and D.G. Mead. 2012. Vector competence of *Culicoides sonorensis* (Diptera: Ceratopogonidae*)* to epizootic hemorrhagic disease virus serotype 7. *Parasitology Vectors* 12: 236-243.

ABSTRACT: *Culicoides sonorensis* (Diptera: Ceratopogonidae) is a vector of epizootic hemorrhagic disease virus (EHDV) serotypes 1 and 2 in North America, where these viruses are well-known pathogens of white-tailed deer (WTD) and other wild ruminants. Although historically rare, reports of clinical EHDV infection in cattle have increased in some parts of the world over the past decade. In 2006, an EHDV-7 epizootic in cattle resulted in economic loss for the Israeli dairy industry. White-tailed deer are susceptible to EHDV-7 infection and disease; however, this serotype is exotic to the U.S. and the susceptibility of *C. sonorensis* to this cattle-virulent EHDV is not known. The objective of the study was to determine if *C. sonorensis* is susceptible to EHDV-7 infection and is a competent vector. To evaluate the susceptibility of *C. sonorensis*, midges were fed on EHDV-7 infected WTD, held at 22 ± 1^{o}C, and processed individually for virus isolation and titration on 4-16 days post feeding (dpf). Midges with a virus titer of ≥ 10(2.7) median tissue culture infective doses (TCID(50))/midge were considered potentially competent. To determine if infected *C. sonorensis* were capable of transmitting EHDV-7 to a host, a susceptible WTD was then fed on by a group of 14-16 dpf midges. From 4-16 dpf, 45% (156/350) of midges that fed on WTD with high titer viremia (>10(7) TCID(50)/ml) were virus isolation-positive, and starting from 10-16 dpf, 32% (35/109) of these virus isolation-positive midges were potentially competent (≥ 10(2.7) TCID(50)/midge). Midges that fed on infected deer transmitted the virus to a susceptible WTD at 14-16 dpf. The WTD developed viremia and severe clinical disease. This study demonstrates that *C. sonorensis* is susceptible to EHDV-7 infection and can transmit the virus to susceptible WTD, thus, *C. sonorensis* should be considered a potential vector of EHDV-7. Together with previous work, this study demonstrates that North America has a susceptible ruminant and vector host for this exotic, cattle-virulent strain of EHDV-7.

Shelley, V., S. Kaiser, E. Shelley, T. Williams, M. Kramer, K. Haman, K. Keel, and H. Barton. 2013. Evaluation of strategies for the decontamination of equipment for *Geomyces destructans*, the causative agent of white nose syndrome (WNS). *Journal of Cave and Karst Studies* 75(1): 1-10.

ABSTRACT: White nose syndrome is an emerging infectious disease that has led to a dramatic decline in cave-hibernating bat species. White nose syndrome is caused by the newly described fungal pathogen *Geomyces destructans*, which infects the ear, muzzle, and wing membranes of bats. Although the exact mechanism by which the fungus causes death is not yet understood, *G. destructans* leads to a high mortality rate in infected animals. While the primary mechanism of infection appears to be bat-to-bat transfer, it is still unclear what role human activity may play in the spread of this pathogen. Here we evaluate the effectiveness of decontamination protocols that can be utilized by speleologists to reduce the likelihood of spreading this dangerous pathogen to naïve bats or uninfected hibernacula. Our results show that pre-cleaning to remove muds and/or sediments followed by the use of commercially available disinfectants can effectively remove *G. destructans* from caving fabrics. Alternatively, immersion in water above 50^{o}C for at least 20 minutes effectively destroys the fungal spores. These results have allowed the development of a

decontamination protocol (http://www.fws.gov/whitenosesyndrome/cavers.html) that, when appropriately followed, can greatly reduce the likelihood of the human mediated transfer of *G. destructans* from an infected to uninfected site.

Shock, B.C., A.J. Birkenheuer, L.L. Patton, C. Olfenbuttel, J. Beringer, D.M. Grove, M. Peek, J.W. Butfiloski, D.W. Hughes, J.M. Lockhart, M.W. Cunningham, H.M. Brown, D.S. Peterson, and M.J. Yabsley. 2012. Variation in the ITS-1 and ITS-2 rRNA genomic regions of *Cytauxzoon felis* from bobcats and pumas in the eastern United States and comparison with sequences from domestic cats. *Veterinary Parasitology* 190(1-2): 29-35.

ABSTRACT: *Cytauxzoon felis*, a tick-borne protozoan parasite, is the causative agent of cytauxzoonosis in domestic cats in the United States. The natural reservoir for this parasite is the bobcat (*Lynx rufus*), which typically does not develop clinical signs. Although not likely important reservoirs, *C. felis* has also been detected in pumas (*Puma concolor*) in Florida and Louisiana. Recent studies suggest that specific genotypes of *C. felis* that circulate in domestic cats may be associated with variable clinical outcomes and specific spatial locations. In the current study, we investigated the intraspecific variation of the *C. felis* internal transcribed spacer (ITS)-1 and ITS-2 rRNA regions from 145 wild felids (139 bobcats and six pumas) from 11 states (Florida, Georgia, Kansas, Kentucky, Louisiana, Missouri, North Carolina, North Dakota, Oklahoma, Pennsylvania, and South Carolina). Unambiguous ITS-1 and ITS-2 data were obtained for 144 and 112 samples, respectively, and both ITS-1 and ITS-2 sequences were obtained for 111 (77%) samples. For the ITS-1 region, sequences from 65 samples collected from wild felids were identical to those previously reported in domestic cats, while the other 79 sequences were unique. *C felis* from 45 bobcats and one puma had ITS-1 sequences identical to the most common sequence reported from domestic cats. Within the ITS-2 region, sequences from 49 bobcats were identical to those previously reported in domestic cats and 63 sequences were unique (with some occurring in more than one bobcat). The most common ITS-2 sequence from domestic cats was also common in wild felids (31 bobcats and a puma). Samples from three pumas from Florida and two bobcats from Missouri had a 40- and 41-bp insert in the ITS-2 similar to one described previously in a domestic cat from Arkansas. Additionally, a previously undescribed 198- or 199-bp insert was detected in the ITS-2 sequence from four bobcats. Collectively, based on combined ITS-1 and ITS-2 sequences, five different genotypes were detected in the wild felids. Genotype ITSa was the most common genotype (11 bobcats and one puma) and fewer numbers of ITSb, ITSe, ITSg, and ITSi were detected in bobcats. These data indicate that, based on ITS-1 and ITS-2 sequences, numerous *C. felis* strains may circulate in wild felids.

Shock, B.L., J.M. Lockhart, A.J. Birkenheuer, and M.J. Yabsley. 2013. Detection of a *Babesia* species in a bobcat from Georgia. *Southeastern Naturalist* 12(1): 243-247.

ABSTRACT: We describe the first detection of a *Babesia* sp. in a *Lynx rufus* (bobcat). The bobcat was from Georgia and was coinfected with *Cytauxzoon felis* and a *Sarcocystis* sp. The *Babesia* species was closely related to *Babesia* sp. "Coco", a parasite previously only detected in *Canis familaris* (domestic dog). The only other *Babesia* sp. in North America that infects felids is a novel *Babesia* species in *Puma concolor coryi* (Florida puma). The low prevalence of this *Babesia* (<1%) in bobcats suggests that they are not the normal host or reservoir and this may have been an incidental infection.

Smith, K.F., M.J. Yabsley, S. Sanchez, C.L. Casey, M.D. Behrens, S.M. Hernandez. 2012. *Salmonella* isolates from wild-caught Tokay geckos (*Gekko gecko*) imported to the U.S. from Indonesia. *Vector-Borne Zoonotic Diseases* 12(7): 575-582.

ABSTRACT: Reptiles account for ~10% of live animal shipments imported to the United States (U.S.), the majority of which are sold in the pet trade. Characterizing *Salmonella* shedding by imported reptiles is of value to public health, the pet industry, and veterinary medicine. Here we report results of a pilot survey of *Salmonella* serotypes isolated from wild-caught Indonesian Tokay geckos (*Gekko gecko*) imported to the U.S. Upon arrival, the geckos were individually housed until a fecal sample was acquired for *Salmonella* culture. The geckos were divided into three groups of variable numbers to investigate density effects. A second group was imported after three months and combined with the previous groups. A total of 88 *Salmonella* isolates were obtained from 110 geckos surveyed, representing 14 serogroups and 17 unique serotypes. Group prevalence ranged from 31-73%. A significant increase in prevalence and a change in serotype richness were detected between the time of import and six months later at necropsy. Six isolates (6.8%) expressed resistance to more than one antibiotic. All *S. enterica* subsp. *enterica* Adelaide isolates were resistant to nalidixic acid and sulfisoxazole, one *S. enterica* subsp. *arizonae* 61:k:z35 isolate was resistant to ampicillin and sulfisoxazole, and another 61:k:z35 isolate was resistant to streptomycin and sulfisoxazole. Forty-three additional isolates expressed resistance only to sulfisoxazole. The mechanisms for increased prevalence and apparent change in serotype richness are unknown, but could be due to stress associated with trade, transport, and captivity, increased transmission from unnaturally high densities, or contact with other species shedding *Salmonella* along the trade route. Future studies to differentiate the physical, social, and physiological effects of trade-related conditions on *Salmonella* shedding and transmission among reptiles will benefit the industry by identifying ways to reduce mortality, and safeguard the individuals handling animals along the transport chain and other species encountered en route.

Smith, P.F., E.W. Howerth, D.L. Carter, E.W. Gray, R. Noblet, R.D. Berghaus, D.E. Stallknecht, and D.G. Mead. 2012. Host predilection and transmissibility of vesicular stomatitis New Jersey virus strains in domestic cattle (*Bos taurus*) and swine (*Sus scrofa*). *BMC Veterinary Research* 8: 183-191.

ABSTRACT: Epidemiologic data collected during epidemics in the western United States combined with limited experimental studies involving swine and cattle suggest that host predilection of epidemic vesicular stomatitis New Jersey virus (VSNJV) strains results in variations in clinical response, extent and duration of virus shedding and transmissibility following infection in different hosts. Laboratory challenge of livestock with heterologous VSNJV strains to investigate potential viral predilections for these hosts has not been thoroughly investigated. In separate trials, and homologous VSNJV strains (NJ82COB and NJ82AZB), and heterologous strains (NJ06WYE and NJOSF [Ossabaw Island, sand fly]) were inoculated into cattle via infected black fly bite. NJ82AZB and NJ06WYE were similarly inoculated into swine. Clinical scores among viruses infecting cattle were significantly different and indicated that infection with a homologous virus resulted in more severe clinical presentation and greater extent and duration of viral shedding. No differences in clinical severity or extent and duration of viral shedding were detected in swine. Differences in clinical presentation and extent and duration of viral shedding may have direct impacts on viral spread during epidemics. Viral transmission via animal-to-animal contact and insect vectored transmission are likely to occur at higher rates when affected animals are presenting severe clinical signs and shedding high concentrations of virus. More virulent viral strains resulting in more severe disease in livestock hosts are expected to spread more rapidly and greater distances during epidemics than those causing mild or inapparent signs.

Tate, C.M., E.W. Howerth, D.G. Mead, V.G. Dugan, M.P. Luttrell, A.I. Sahora, U.G. Munderloh, W.R. Davidson, and M.J. Yabsley. 2013. *Anaplasma odocoilei* sp. nov (Family Anaplasmataceae) from white-tailed deer (*Odocoileus virginianus*). *Ticks and Tick-Borne Diseases* 4(1-2): 110-119.

ABSTRACT: Recently, an undescribed *Anaplasma* sp. (also called *Ehrlichia*-like sp. or WTD agent) was isolated in ISE6 tick cells from captive white-tailed deer. The goal of the current study was to characterize this organism using a combination of experimental infection, morphologic, serologic, and molecular studies. Each of six experimentally inoculated white-tailed deer fawns (*Odocoileus virginianus*) became chronically infected (100+ days) with the *Anaplasma* sp. by inoculation of either infected whole blood or culture. None of the deer showed evidence of clinical disease, but three of the six deer evaluated had multiple episodes of transient thrombocytopenia. Light microscopy of Giemsa-stained, thin blood smears revealed tiny, dark, spherical structures in platelets of acutely infected deer. *Anaplasma* sp. was detected in platelets of inoculated deer by polymerase chain reaction, transmission electron microscopy, immunohistochemistry, and in situ hybridization. Five of six deer developed antibodies reactive to *Anaplasma* sp. antigen, as detected by indirect fluorescent antibody testing. Phylogenetic analyses of 16S rRNA, groESL, and gltA sequences confirmed the *Anaplasma* sp. is related to *A. platys*. Two attempts to transmit the *Anaplasma* sp. between deer by feeding *Amblyomma americanum*, a suspected tick vector, were unsuccessful. Based on its biologic, antigenic, and genetic characteristics, this organism is considered a novel species of Anaplasma, and the name *Anaplasma odocoilei* sp. nov. is proposed with UMUM76(T) (=CSUR-A1) as the type strain.

Xu, B., M. Madden, D.E. Stallknecht, T.W. Hodler, and K.C. Parker. 2012. Spatial and spatial-temporal, clustering analysis of hemorrhagic disease in white-tailed deer in the southeastern USA; 1980-2003. *Preventive Veterinary Medicine* 106(3-4): 339-347.

ABSTRACT: We used the space-time K function and Kulldorff's scan statistic to analyze the spatial and spatial-temporal clustering of hemorrhagic disease (HD) in white-tailed deer in Alabama, Georgia, North Carolina, South Carolina, and Tennessee. The HD occurrence data were binary presence/absence data acquired annually on a county basis from 1980 to 2003. Space-time K function was employed to globally examine the existence of spatial-temporal clustering in the HD data. Three approaches of Kulldorff's scan statistic, i.e., spatial clustering analysis for the entire period, spatial-temporal clustering analysis, and spatial clustering analysis by individual years, were applied to detect potential HD clusters. Statistically significant spatial clusters and spatial-temporal clusters were detected in the five southeastern states during the 24-year study period. Some clusters were observed in multiple years. Clusters were most evident in west Alabama, south Alabama, central South Carolina, and along the border between South Carolina and North Carolina. The identification of HD clusters may provide a means to better understand the causal factors related to the HD outbreaks. Results also have potential application in improving or designing effective surveillance programs for this disease.

Yabsley, M.J., and B.C. Shock. 2013. Natural history of zoonotic *Babesia*: role of wildlife reservoirs. *International Journal for Parasitology: Parasites and Wildlife* 2:18-31.

ABSTRACT: Babesiosis is an emerging zoonotic disease on all inhabited continents and various wildlife species are the principal reservoir host for zoonotic *Babesia* species. The primary vectors of *Babesia* are Ixodid ticks, with the majority of zoonotic species being transmitted by species in the genus *Ixodes*. Species of *Babesia* vary in their infectivity, virulence and pathogenicity for people. Various factors (e.g., increased interactions

between people and the environment, increased immunosuppression, changes in landscape and climate, and shifts in host and vector species abundance and community structures) have led to an increase in tick-borne diseases in people, including babesiosis. Furthermore, because babesiosis is now a reportable disease in several states in the United States, and it is the most common blood transfusion-associated parasite, recognized infections are expected to increase. Because of the zoonotic nature of these parasites, it is essential that we understand the natural history (especially reservoirs and vectors) so that appropriate control and prevention measures can be implemented. Considerable work has been conducted on the ecology of *Babesia microti* and *Babesia divergens*, the two most common causes of babesiosis in the United States and Europe, respectively. However, unfortunately, for many of the zoonotic *Babesia* species, the reservoir(s) and/or tick vector(s) are unknown. We review the current knowledge regarding the ecology of *Babesia* among their reservoir and tick hosts with an emphasis on the role of wildlife as reservoirs. We hope to encourage the molecular characterization of *Babesia* from potential reservoirs and vectors as well from people. These data are necessary so that informed decisions can be made regarding potential vectors and the potential role of wildlife in the ecology of a novel *Babesia* when it is detected in a human patient.

Yabsley, M.J., S.E. Clay, S.E.J. Gibbs, M. Cunningham, and M.G. Austel. 2013. Morphologic and molecular characterization of a *Demodex* (Acari: Demodicidae) species from white-tailed deer (*Odocoileus virginianus*). *ISRN Parasitology,* Article 342918, 7 pages.

ABSTRACT: *Demodex* mites, although usually nonpathogenic, can cause a wide range of dermatological lesions ranging from mild skin irritation and alopecia to severe furunculosis. Recently, a case of demodicosis from a white-tailed deer (*Odocoileus virginianus*) revealed a *Demodex* species morphologically distinct from *Demodex odocoilei*. All life cycle stages were considerably larger than *D. odocoilei* and although similar in size to *D. kutzeri* and *D. acutipes* from European cervids, numerous morphometrics distinguished the four species. Adult males and females were 209.1 ± 13.1 and 225.5 ± 13.4 µm in length, respectively. Ova, larva, and nymphs measured 65.1 ± 4.1, 124.9 ± 11.6, and 205.1 ± 19.4 µm in length, respectively. For phylogenetic analyses, a portion of the 18S rRNA gene was amplified and sequenced from samples of the WTD *Demodex* sp., two *Demodex* samples from domestic dogs, and *Demodex ursi* from a black bear. Phylogenetic analyses indicated that the WTD *Demodex* was similar to *D. musculi* from laboratory mice. A partial sequence from *D. ursi* was identical to the WTD *Demodex* sequence; however, these two species can be differentiated morphologically. This paper describes a second *Demodex* species from white-tailed deer and indicates that 18S rRNA is useful for phylogenetic analysis of most *Demodex* species, but two morphologically distinct species had identical partial sequences. Additional gene targets should be investigated for phylogenetic and parasite-host association studies.

<u>In Press</u>:

Boone, S.S., S.J. Divers, A.C. Camus, D.L. Peterson, C.A. Jennings, J.L. Shelton, and S.M. Hernandez. Pathologic and physiologic effects associated with long-term intracoelomic transmitters in captive Siberian sturgeon (*Acipenser baerii*). *North American Journal of Fisheries Management.*

Cottrell, W., M.K. Keel, J. Brooks, D.E. Mead, and J. Phillips. First report of clinical disease associated with canine distemper virus in a wild black bear. *Journal of Wildlife Diseases*.

Gonzales, A.V., S.M. Hernandez, S.L. Boone, E.K. Lipps, S. Shersthra, and M.J. Yabsley. Spatial, temporal, and intraspecific difference of haemoparasite infection and relevant physiological parameters of wild birds in Georgia, USA. *International Journal for Parasitology: Parasites and Wildlife.*

Keeler, S.P., M.J. Yabsley, J.M. Fox, S.N. McGraw, and S.M. Hernandez. *Isospora troglodytes* n. sp. (Apicomplexa: Eimeriidae), a new coccidian species from wrens of Costa Rica. *Parasitology Research*.

Kjos, S.A., P. Marcet, M.J. Yabsley, U. Kitron, K. Snowden, K. Logan, J. Barnes, and E. Dotson. Identification of blood meal sources and *T. cruzi* infection in triatomine bugs (Hemiptera: Reduviidae) from residential settings in south central Texas. *Journal of Medical Entomology*.

Lebarbenchon, C., J.D. Brown, and D.E. Stallknecht. Recent evolutionary history of avian H7 and N9 influenza virus subtypes in Eastern Asia. *Emerging Infectious Diseases*.

Levine, R.S., D.G. Mead, and U.D. Kitron. Limited spillover to humans from West Nile virus viremic birds in Atlanta, Georgia. *Vector-Borne and Zoonotic Diseases*.

Merkens, L.R., L.A. Shender, M.J. Yabsley, B.S. Shock, F.A. Chinchilla, J. Suarez, K.V.K. Gilardi. White-nosed coatis (*Nasua narica*) are a potential reservoir of *Typanosoma cruzi* and other potentially zoonotic pathogens in Monteverde, Costa Rica. *Journal of Wildlife Diseases.*

McGuire, J.L., E.A. Miller, T.M. Norton, B.L. Raphael, J.S. Spratt, and M.J. Yabsley. Intestinal parasites of the gopher tortoise (*Gopherus polyphemus*) from eight populations in Georgia. *Parasitology Research*.

McGuire, J.L., S.M. Hernandez, L.L. Smith, and M.J. Yabsley. Safety and utility of an anesthetic protocol for the collection of biological samples from gopher tortoises (*Gopherus polyphemus*). *Wildlife Society Bulletin.*

Munk, B.A., J.C. Turner, and M.K. Keel. Mediastinal teratoma in a free-ranging American black bear (*Ursus americanus*). *Microbiology and Immunology*.

Nemeth, N., M. Ruder, R. Gerhold, J. Brown, B. Munk, P. Oesterle, S. Kubiski, and K. Keel. Demodectic mange, dermatophilosis, and other infectious dermatologic diseases in free-ranging white-tailed deer (*Odocoileus virginianus*) in the southeastern United States, 1977-2012. *Veterinary Pathology*.

Ramey, A., E. Spackman, J. Yeh, G. Fujita, K. Konishi, K. Uchida, J.A. Reed, B.R. Wilcox, J.D. Brown, and D.E. Stallknecht. Antibodies to H5 subtype avian influenza virus and Japanese encephalitis virus in northern pintails (*Anas acuta*) sampled in Japan. *Japanese Journal of Veterinary Research.*

Submitted to Journal:

Allison, A.B., D.G. Mead, G.F. Palacios, R.B. Tesh, and E.C. Holmes. Gene duplication and phylogeography of North American members of the Hart Park serogroup of avian rhabdoviruses. *Journal of Virology*.

Barton, H., P. Rohani, D. Stallknecht, J. Brown, and J. Drake. Subtype diversity of reassortment potential for co-circulating avian influenza viruses at a diversity hotspot. *Ecology Letters.*

Conrad, J., J. Norman, A. Rodriguez, P. Dennis, R. Arguedas, C. Jimenez, M.J. Yabsley, and S.M. Hernandez. Survey of domestic pets for exposure to and infection with selected pathogens and the implications for wild carnivore and human health in the San Luis region of Costa Rica. *EcoHealth*.

Fankhauser, M., D. Sprenger, R. Kelly, S. Mukherjee, J. Huang, D.B. Huddleston, T.F. Jones, D.G. Mead, and A.C. Moncayo. Spatial-temporal analysis of the relationship between Flanders and West Nile viruses. *PLoS One*.

Fritzen, C., E. Mosites, R.D. Applegate, S.R. Telford, J. Huang, M.J. Yabsley, L.R. Carpenter, J.R. Dunn, and A.C. Moncayo. Ecological investigation following the first human case of babesiosis in Tennessee. *Emerging Infectious Diseases.*

Gargano, L.M., J. Engel, G.C. Grey, K. Howell, T. Jones, W.K. Milhous, J.G. Morris, D.G. Mead, C. Rubin, T.R. Unnasch, C.W. Woods, and J.M. Hughes. Arboviral diseases in the southeastern United States. *Emerging Infectious Diseases*.

Hernandez, S.M., B.J. Mattson, R.J. Cooper, and C.R. Carroll. Seasonal dynamics of avian communities in secondary forest and shade-coffee plantations of the Monteverde region, Costa Rica. *Biological Conservation*.

Kistler, W.M., J.D. Brown, and M.J. Yabsley. First report of *Angiostrongylus vasorum* and *Hepatozoon canis* in a red fox (*Vulpes vulpes*) from the United States. *Veterinary Parasitology*.

Ladd, K., K. Legleu, and K. Keel. Mortality among ground foraging tree swallows due to collisions with vehicles. *Southeastern Naturalist*.

Lebarbenchon, C., J.D. Brown, E. Spackman, W. Kistler, M.P. Luttrell, M. Pantin-Jackwood, and D.E. Stallknecht. Evaluation of a commercial enzyme-linked immunosorbent assay for detection of low- and high-pathogenicity H5 Influenza A antibodies. *Journal of Veterinary Diagnostic Investigation.*

Lund, A., J. McMillan, R. Kelly , S. Jabbarzadeh, D.G. Mead, T.R. Burkot, U. Kitron, and G. M. Vazquez Prokopec. Long term impacts of Combined Sewer Overflow remediation on water quality, mosquito population dynamics and West Nile virus amplification. *Environmental Health Perspectives*.

Mertins, J.W., W. Gaston, and J.L. Corn. First record of chewing lice, *Damalinia (Tricholipeurus) lipeuroides* and *D. parallela* (Phthiraptera: Trichodectidae), on white-tailed deer, *Odocoileus virginianus* (Mammalia: Cervidae), in the U.S. Virgin Islands. *Caribbean Journal of Science.*

Roche, B., J.M. Drake, T. Bedford, J.D. Brown, D.E. Stallknecht, and P. Rohani. Genetic diversity of influenza viruses is jointly determined by the ecology of transmission and host demography. *Proceedings of the National Academy of Sciences.*

Roellig, D.M., L.A. Gomez-Puerta, D.G. Mead, J. Pinto, J. Ancca, M.Z. Levy, C. Bern, R.H. Gilman, and V.A. Cama. Novel hemi-nested PCR and RFLP methodologies for identifying

blood meals of the Chagas disease vector, *Triatoma infestans*. *PLoS Neglected Tropical Diseases.*

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. Justin D. Brown: Academic Editor, *PLos One*; Member, Student Awards Committee, Wildlife Disease Association; Guest Editor, *Proceedings of the 8th International Symposium on Avian Influenza*.

Dr. Joseph L. Corn: Chair, Subcommittee on Pseudorabies and Brucellosis in Feral Swine, United States Animal Health Association (USAHA); Member, Committee on Parasitic Diseases, USAHA; Member, Committee on Public Health and Rabies, USAHA; Member, Committee on Foreign and Emerging Diseases, USAHA; Member, Committee on Wildlife Diseases, USAHA; Member, Committee on Bluetongue and Related Orbiviruses, USAHA; Member, Wildlife Diseases Working Group, The Wildlife Society; Member, Invasive Species Working Group, The Wildlife Society; Member, Working Group on Feral Swine, National Institute for Mathematical and Biological Synthesis; Georgia Feral Hog Working Group; Feral Hog Community of Practice, eXtension, Land-Grant University System; Caribvet Working Group on Ticks and Tick-borne Diseases.

Dr. John R. Fischer: Member, U.S. Secretary of Agriculture Advisory Committee on Animal Health; Member, Wildlife Disease Working Group, World Animal Health Organization (OIE); Chair, Committee on Wildlife Diseases, U.S. Animal Health Association (USAHA); Member, Board of Directors representing the Association of Fish and Wildlife Agencies (AFWA), USAHA; Member, Tuberculosis Committee, USAHA; Member, International Standards Committee, USAHA; Member, Captive Wildlife and Alternative Livestock Committee, USAHA; Vice Chair, Fish and Wildlife Health Committee, AFWA; Vice Chair, Steering Committee, National Fish and Wildlife Health Initiative; Member, Sustainable Use of Wildlife Committee, AFWA; Chair, AFWA CWD Working Group; Member, Wildlife Diseases Working Group, The Wildlife Society; Member, Board of Directors, Arcadia Wildlife Preserve Foundation, Inc.; Professional Member, Boone and Crockett Club.

Dr. Sonia M. Hernandez: Chair, Education Committee, American College of Zoological Medicine; Member, Infectious Disease Committee and Wildlife Health Committee, American Association of Zoo Veterinarians; Advisory Board Member of Wildlife Veterinary Section of Wildlife Disease Association; Chair, International Veterinary Medicine Committee, Study Abroad Risk Assessment Board, The University of Georgia; Member, Stoddard-Dutton Conservation/Ornithology Award Committee, The University of Georgia; Member, Admissions Committee, College of Veterinary Medicine, The University of Georgia; Member, *Ad Hoc* Committee, IAUCUC, The University of Georgia; Council Representative, The University of Georgia.

Dr. Daniel G. Mead: Member, Committee on Wildlife Diseases, USAHA; Member, Committee on Parasitic Diseases, USAHA; Member, Committee on Bluetongue and Related

Orbiviruses, USAHA; Member, West Nile Virus Working Group, State of Georgia; Member, DVM-MPH Committee, College of Veterinary Medicine, The University of Georgia; Co-Chair, Biosafety Community Liaison Committee, Office for the Vice President of Research, The University of Georgia; AHRC Users Committee, College of Veterinary Medicine, The University of Georgia.

Dr. David E. Stallknecht: Assistant Editor, *Journal of Wildlife Diseases*; Member, Committee on Wildlife Diseases, USAHA; Member, Committee on Bluetongue and Related Orbiviruses, USAHA; Member, Scholarship and Appeals Committee, College of Veterinary Medicine, The University of Georgia.

Dr. Michael J. Yabsley: Member, Information Transfer Committee, Wildlife Disease Association (WDA); Member, Long-Term Investment Committee, WDA; Member, Membership Committee, WDA; Member, Wildlife Diseases Working Group, The Wildlife Society; Member, Diseases Task Team Southeast Partners in Amphibian and Reptile Conservation; Member, Meritorious Service Award Committee, Southeastern Society of Parasitologists; President, Georgia Chapter of The Wildlife Society; Member, R. Barclay McGhee Memorial Lectureship Committee, American Society of Parasitologists.

TRAVEL ASSOCIATED WITH SCWDS ACTIVITIES

July 5-7, 2012
Brown, Slusher, and Thomas. Jacksonville, FL. To collect samples from gulls for avian influenza virus research.

July 5-August 10, 2012
Ballard. Madison, WI. To assist with the common eider inoculation trial being conducted at the National Wildlife Health Center.

July 11-13, 2012
Brown and Keel. Bellefonte, PA. To conduct a Wildlife Disease Workshop at the H.R. Stackhouse School at the request of the Pennsylvania Game Commission.

July 21-28, 2012
Kistler. Lyon, France. To attend the 61st Wildlife Disease Association Annual Meeting and give a presentation entitled "H5 Avian Influenza Specific Antibodies in Mute Swans from the United States."

July 23-26, 2012
Shaw. Fort Myers, FL. To transport supplies used in exotic tick surveillance from the Fort Myers, Florida, field office to SCWDS.

July 25-August 6, 2012
Edwards de Vargas. Athens, GA. To transport supplies used in exotic tick surveillance from the Fort Myers, Florida, field office to SCWDS.

July 27-August 1, 2012
Wilcox. Middle River, MN. To collect serum samples and cloacal swabs from birds for avian influenza virus research.

July 28-August 1, 2012
Brown, Poulson, and Stallknecht. New York City, NY. To attend the 6th Annual Centers of Excellence for Influenza Research and Surveillance (CEIRS) Network Meeting.

July 30-August 3, 2012
Carter, Chillag, Munk, Slusher, and Thomas. Brunswick, GA. To conduct deer herd health evaluations at Jekyll Island and Harris Neck National Wildlife Refuge.

July 31-September 15, 2012
Murray. Big Pine Key, FL. To collect samples from Key deer for Johnes surveillance.

August 5-11, 2012
Kistler. Shepherdstown, WV. To attend the 2nd Annual International Workshop on Malaria and Related Haemosporidian Parasites of Wildlife meeting and give a presentation entitled "Phylogenetic Analysis of Malaria Parasites Circulating in North American Black Ducks."

August 6-18, 2012
Shaw. Clayton, Demopolis, Mobile, Jackson, and Tallassee, AL; Carrabelle, Crestview, Panama City, and Pensacola, FL; and Bainbridge, Colquitt, and Vienna, GA. To collect *Culicoides*, known vectors of EHD, at Barbour, David K. Nelson, Fred T. Stimpson, Mobile-Tensaw, and Yates Lake West Wildlife Management Areas in Alabama; Ecofina Creek, Escambia River, Tate's Hell, and Yellow River Wildlife Management Areas in Florida; and Flint River, Lake Seminole, and Mayhaw Wildlife Management Areas in Georgia.

August 6-18, 2012
Wlodkowski. Beggs, Deville, LaPlace, Liddieville, Sherburne, and Swampers, LA; Howison, Lucdale, Mt. Olive, Ovett, Shipland, and Water Valley, MS. To collect *Culicoides*, known vectors of EHD, at Big Lake, Boeuf, Dewey Wills, Maurapas Swamp, Sherburne, Swampers, and Thistlethwaite Wildlife Management Areas in Louisiana; Evans Property in Mississippi; and Caston Creek, Chickasawhay, Little Biloxi, Pascagoula River, and Shipland Wildlife Management Areas in Mississippi.

August 14-18, 2012
Brown. Vancouver, British Columbia, Canada. To attend the 5th North American Ornithological Conference (NAOC-V) and give a presentation entitled "Understanding the Avian Influenza Virus in Gulls."

August 29-September 5, 2012
Carter. Middle River, MN. To collect samples from waterfowl and mammals for avian influenza epidemiology studies.

August 29-September 24, 2012
Oesterle and Wilcox. Middle River, MN. To collect samples from waterfowl and mammals for avian influenza epidemiology studies.

September 4-16, 2012
Shaw. Clayton, Demopolis, Rockville, and Tallassee, AL; Milton, Panama City, and Pensacola, FL; and Bainbridge, Colquitt, and Vienna, GA. To collect *Culicoides*, known vectors of EHD, at Barbour Wildlife Management Area (WMA), Mobile-Tensaw WMA, David K. Nelson WMA, Fred T. Stimpson WMA, and Yates Lake West WMA in Alabama; Econfina Creek WMA, Escambia River WMA, and Yellow River WMA in Florida; and Flint River WMA, Lake Seminole WMA, and Mayhaw WMA in Georgia.

September 5, 2012
Mead. Riverdale, MD. To meet with senior officials at USDA to discuss the use of the Animal Health Research Center at The University of Georgia.

September 9-13, 2012
Fischer. Hilton Head, SC. To attend the Association of Fish and Wildlife Agencies (AFWA) 2012 North American Wildlife and Natural Resources Conference and Co-Chair the Fish and Wildlife Health Committee.

September 11-14, 2012
McElwee. Hilton Head, SC. To attend the Association of Fish and Wildlife Agencies (AFWA) 2012 North American Wildlife and Natural Resources Conference and record minutes for the Fish and Wildlife Health Committee.

September 12-23, 2012
Wlodkowski. Various WMAs in LA & MS. To collect *Culicoides*, known vectors of EHD, various wildlife management areas in Louisiana and Mississippi.

September 12-30, 2012
Carter. Sulphur, LA and Winnie, TX. To collect samples from hunter-killed green-winged and blue-winged teal in Louisiana and Texas for avian influenza virus research.

October 1-9, 2012
Shaw. Various WMAs in AL, FL, & GA. To collect *Culicoides*, known vectors of EHD, at various wildlife management areas in Alabama, Florida, and Georgia.

October 3-11, 2012
Cleveland. Various WMAs in LA and MS. To collect *Culicoides*, known vectors of EHD, at various wildlife management areas in Louisiana and Mississippi.

October 6-9, 2012
Fischer. Hot Springs, AR. To attend the 66th Annual Conference of the Southeastern Association of Fish and Wildlife Agencies (SEAFWA).

October 9-10, 2012
Stallknecht. Washington, DC. To attend the Colloquium on Influenza for the American Academy of Microbiology, ASM.

October 9-12, 2012
Fischer. Washington DC. To attend the National Animal Wildlife Health Strategy Workshop and give a presentation entitled "Update on the National Fish and Wildlife Health Initiative" at the request of the U.S. Department of the Interior.

October 13-21, 2012
Kistler and Shock. Kunming City, Yunnan Province, China. To attend the Next Generation Sequencing Workshop and the EcoHealth 2012 Meeting and present research findings at the conference.

October 15-18, 2012
Fischer. Portland, OR. To attend The Wildlife Society 19th Annual Conference and give the Al Franzman presentation entitled "Reducing Risk Factors for Disease in Wildlife" at the request of the American Association of Wildlife Veterinarians.

October 19-22, 2012
Brown. Greensboro, NC. To attend the 116th United States Animal Health Association and 55th American Association of Veterinary Laboratory Diagnosticians Annual Meeting and give a presentation entitled "Identification of Lymphoproliferative Disease Virus in Wild Turkeys (*Meleagris gallopavo*) in the United States" to the Committee on Wildlife Diseases and a presentation entitled "Identification of Lymphoproliferative Disease Virus in Wild Turkeys (*Meleagris gallopavo*) in the United States" at the AAVLD meeting.

October 19-25, 2012
Fischer. Greensboro, NC. To attend the 116th United States Animal Health Association and 55th American Association of Veterinary Laboratory Diagnosticians Annual Meeting, serve as Chair of the USAHA Committee on Wildlife Diseases and represent the Association of Fish and Wildlife Agencies on the USAHA Board of Directors.

October 19-26, 2012
Hernandez. Oakland, CA. To attend the American Association of Zoo Veterinarians Annual Conference and give presentations entitled "*Salmonella* of Free-ranging Mesomammals," "Ecoimmunology: *Bufo marinus* as a Case Study, Wildlife Disease Investigation," and "Global Emergent Wildlife Diseases."

October 20-22, 2012
Munk. Greensboro, NC. To attend the 116th United States Animal Health Association and 55th American Association of Veterinary Laboratory Diagnosticians Annual Meeting and give presentations entitled "Antleromas and Hummel Deer: Lessons on Antlerogenesis from Free-ranging White-tailed Deer" and "Scoliosis in Three Gopher Tortoises from Baker County, Georgia."

October 21-22, 2012
Mead. Greensboro, NC. To attend the 116th United States Animal Health Association Annual Meeting and give presentations entitled "Hemorrhagic Disease in 2012" to the Committee on Wildlife Diseases and "SCWDS HD Update" to the Committee on Bluetongue and Related Orbiviruses.

October 21-25, 2012
Corn. Greensboro, NC. To attend the 116th United States Animal Health Association Annual Meeting, serve as Chair of the Feral Swine Subcommittee on Brucellosis and Pseudorabies and give a presentation entitled "The National Feral Swine Mapping System," and give a presentation entitled "SCWDS Tick Surveillance in Texas" to the Committee on Parasitic Diseases.

October 25-26, 2012
Corn. Raleigh, NC. To meet with researchers at North Carolina State University to discuss collaborative studies on diseases in feral swine.

November 2-12, 2012
Cleveland and Shaw. McAllen, TX. To collect exotic tick surveillance at Las Palomas, James Daughtrey, and Chaparral Wildlife Management Areas in Texas.

November 11-16, 2012
Fischer. Paris, France. To attend the annual meeting of the Wildlife Disease Working Group of the World Organisation for Animal Health (OIE) and give a presentation entitled "Wildlife Disease Occurrences in the United States during 2012."

November 13, 2012
Cleveland. Milledgeville, GA. To pick-up a white-tailed deer from Georgia Department of Natural Resources personnel and transport to SCWDS for necropsy.

December 2-4, 2012
Brown. Ft. Collins, CO. To attend Research and Policy for Infectious Disease Dynamics (RAPIDD) Meeting on Avian Influenza and give presentations entitled "Understanding the Role of Aquatic Environments in the Ecology of Avian Influenza" and "Low Pathogenic Avian Influenza in Ducks."

December 3-4, 2012
Corn. Jekyll Island, GA. To attend the annual meeting of Georgia Farm Bureau and give a presentation entitled "Feral Swine Distribution and Disease Issues."

December 5, 2012
Slusher and Thomas. Clarks Hill, SC. To collect American coots for avian vacuolar myelinopathy (AVM) surveillance.

January 7-18, 2013
Fischer. Paris, France. To attend a meeting of an OIE *Ad hoc* Group revising the Brucellosis chapter of the OIE *Terrestrial Animal Health Code* as a wildlife health expert, and to Chair a meeting of an OIE *Ad hoc* Group drafting a new chapter on Validation of Diagnostic Tests for Wildlife for the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* at the request of the World Organisation for Animal Health (OIE).

January 19-21, 2013
Carter and Poulson. Fernandia and Jacksonville, FL. To collect ruddy turnstone samples for avian influenza virus surveillance and to search for additional sites to conduct surveillance in 2013.

February 4-7, 2013
Fischer. Washington, DC. To attend a meeting of the National Wildlife and Hunting Heritage Conservation Council and give a presentation entitled "Hemorrhagic Disease – 2013 Update" at the request of the U.S. Department of the Interior.

February 5-8, 2013
Fojtik and Wilcox. Winnie, TX. To collect samples from snow geese for avian influenza virus surveillance.

February 11-12, 2013
Mead. Atlanta, GA. To attend the Arboviral Infections in the Southeast USA, 2012 meeting at the request of the Biological Emergency Preparedness and Response Program, Southeast Regional Center of Excellence for Emerging Infections and Biodefense and give a presentation entitled "WNV in Georgia: Experiences and Perspectives."

February 18-19, 2013
Slusher. Atlanta, GA. To obtain a research visa from the Consulate General of Brazil in Atlanta.

February 19-20, 2013
Ballard and Fischer. Columbiana, AL. To conduct a Wildlife Workshop for personnel with the Alabama Department of Conservation and Natural Resources, Division of Wildlife and Freshwater Fisheries.

February 20-March 6, 2013
Slusher. Sao Luis, Maranhao, Brazil. To assist with equipping ruddy turnstones with geolocaters to gain a better understanding of migration patterns as part of the NIH grant.

February 27-March 12, 2013
Davis-Fields, Fojtik, Oesterle, and Wilcox. Winnie, TX. To collect samples from waterfowl for avian influenza virus surveillance.

February 28-March 6, 2013
Carter. Anahuac, TX. To collect samples from blue-winged teal for avian influenza virus surveillance.

February 28-March 17, 2013
Ramey. Winnie, TX. To collect samples from waterfowl for avian influenza virus surveillance.

March 1, 2013
Fischer. Conyers, GA. To attend a One Health Meeting at the request of the USDA-APHIS-Veterinary Services.

March 7-8, 2013
Corn. Conyers, GA. To attend a USDA-APHIS-Feral Swine Brucellosis Project Meeting.

March 7-10, 2013
Carter and Poulson. Fernandia Beach and Jacksonville, FL. To collect samples from ruddy turnstones for avian influenza virus surveillance.

March 13-15, 2013
Brown, Poulson, and Stallknecht. Minneapolis, MN. To attend the annual Minnesota Centers of Excellence for Influenza Research and Surveillance (MCEIRS) Investigators meeting at the University of Minnesota.

March 13-17, 2013
Fojtik. Grand Chenier, LA. To collect samples from waterfowl at Rockefeller State Wildlife Refuge for avian influenza virus surveillance.

March 14-24, 2013
Vigil. Gainesville, FL. To meet with Dr. Bill Grogan with the Florida State Collection of Arthropods for assistance with identification of insect of species.

March 25-30, 2013
Fischer. Washington, DC. To attend the 78th North American Wildlife and Natural Resources Conference and the Boone and Crockett Club Meeting, and to serve as Vice Chair of the AFWA Committee on Fish and Wildlife Health.

April 6-8, 2013
Carter and Poulson. Jacksonville, FL. To collect samples from ruddy turnstones for avian influenza virus surveillance.

April 22-24, 2013
Kistler and Stallknecht. Nebraska City, NE. To attend the Annual Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD) meeting.

April 24, 2013
Beck, Dolinski, Fojtik, and Wilcox. Watkinsville, GA. To collect samples from small birds for avian influenza virus and trichomoniasis research.

April 26-29, 2013
Fojtik and Slusher. Kiawah Island, SC. To collect samples from ruddy turnstones for avian influenza virus surveillance and assist with geolocators.

April 28-May 1, 2013
Fischer. Washington, DC. To attend the federal budget briefings at the offices of the Association of Fish and Wildlife Agencies.

May 4-7, 2013
Fischer. Biloxi, MS. To attend the annual Spring Directors' meeting of the Southeastern Association of Fish and Wildlife Agencies.

May 5-June 1, 2013
Ballard. Nova Scotia, Canada. To collect serum samples from common eider as part of the Wellfleet Bay virus research.

May 12-23, 2013
Wilcox. Reeds Beach, NJ. To collect samples from shorebirds for avian influenza virus research and provide assistance equipping ruddy turnstones with geolocators.

May 12-30, 2013
Fojtik and Slusher. Reeds Beach, NJ. To collect samples from shorebirds for avian influenza virus research and provide assistance equipping ruddy turnstones with geolocators.

May 13-16, 2013
Stallknecht. Manhattan, KS. To attend the USDA-ARS Gap Analysis and Countermeasures Assessment for Orbivirus Workshop.

May 20-23, 2013
Ramey and Stallknecht. Reeds Beach, NJ. To collect samples from shorebirds for avian influenza virus research and provide assistance equipping ruddy turnstones with geolocators.

May 20-30, 2013
Thomas. Reeds Beach, NJ. To collect samples from shorebirds for avian influenza virus research and provide assistance equipping ruddy turnstones with geolocators.

May 28-29, 2013
Brown. Loretto, TN. To attend a town meeting with Tennessee Wildlife Resources Agency personnel to discuss wild turkey diseases.

May 30, 2013
Mead. Riverdale, MD. To meet with USDA senior officials to discuss the use of the Animal Health Research Center at UGA.

June 2-4, 2013
Fischer. Frederick and Riverdale, MD. To attend the regional meeting on CWD management and give a presentation entitled "Update on CWD in Wild and Captive Cervids in the United States" and to meet with USDA-APHIS-Veterinary Services personnel coordinating the cooperative agreement with SCWDS.

June 5, 2013
Corn. Conyers, GA. To attend a Georgia One Health Meeting and give a presentation entitled "Feral Hog Distribution and Disease Issues."

June 10-13, 2013
Shaw. Boston, MA. To conduct exotic tick surveillance on Calf Island in Boston Harbor.

June 10-15, 2013
Coker, Cooper, Gabriel, Stanford, Sumner, and Wlodkowski. Danielsville, GA. To collect various species of aquatic turtles for disease surveillance. The data collected will be used to determine presence and prevalence of haemogregarines in aquatic turtles at Reservoir 17 in Madison County.

June 12-13, 2013
Fischer. Tampa, FL. To attend a meeting of the Florida Fish and Wildlife Conservation Commission and give a presentation entitled "Chronic Wasting Disease of Deer and Elk."

June 22-26, 2013
Fischer. Lexington, KY. To attend the Midwest Association of Fish and Wildlife Agencies Annual Directors' Meeting and give a presentation entitled "Current Wildlife Health Issues."

June 25-30, 2013
Shock and Yabsley. Quebec City, Quebec, Canada. To attend the 88th Annual American Society of Parasitologists Meeting. Ms. Shock to give a presentation entitled "Utility of Testing Blood-fed and Questing Ticks for the Identification of Novel Vertebrate Hosts and Novel Vectors." Dr. Yabsley will present data on three research projects.

FINANCIAL STATEMENT FOR FISCAL YEAR 2012-2013

STATE CONTRACT

RF SCWD CONSRTM FISCH – 10-21-RR694-109:

CARRY-OVER FROM CONTRACT PERIOD - ENDING JUNE 30, 2012		$360,645.40
NEW CONTRACT FUNDS - JULY 1, 2012 - JUNE 30, 2013		$463,730.00
TOTAL FUNDS AVAILABLE - JULY 1, 2012 - JUNE 30, 2013		$824,375.40
EXPENDITURES (7/01/12 - 6/30/13):		
Personal Services	$89,086.47	
Staff Benefits	$23,701.15	
Operating Expenses	$178,912.91	
Equipment Expenses	-0-	
Travel	$5,651.35	
TOTAL EXPENDITURES	$297,351.88	$297,351.88
UNENCUMBERED CARRY-OVER INTO CONTRACT PERIOD JULY 1, 2013 - JUNE 30, 2014		$527,023.52