SCWDS BRIEFS

A Quarterly Newsletter from the

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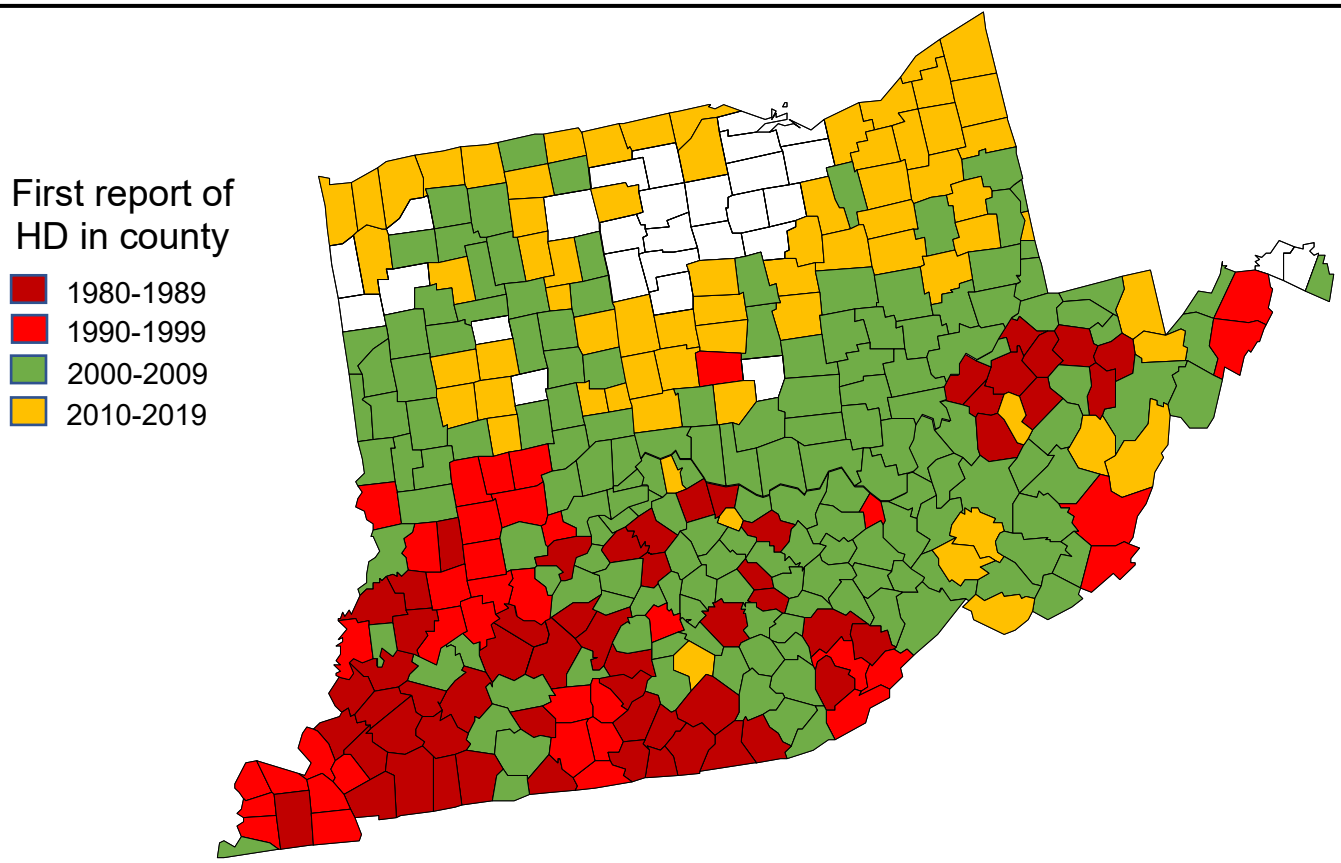

Figure 1. First reports of HD by state fish and wildlife agencies by decade from 1980 to 2019. Over most of this area, HD was rarely reported prior to 2000.

Working Together: The 40th Anniversary of the National Hemorrhagic Disease Survey

Forty years ago, in 1980, Dr. Victor Nettles launched an annual survey designed to document and better understand the distribution and annual patterns of hemorrhagic disease (HD) in the Southeast. Two years later (1982), this survey was expanded to include the entire United States, and its longevity and success can be attributed to a simple but informative design and the dedicated reporting of state fish and wildlife agency personnel. During these 40 years, not a single state agency failed to report their annual HD activity. The original survey was based on four criteria. These included: 1) sudden, unexplained, high mortality during the late summer and early fall; 2) necropsy diagnosis of HD as rendered by a trained wildlife biologist, a diagnostician at a State Diagnostic Laboratory or Veterinary College, or by SCWDS personnel; 3) detection of epizootic hemorrhagic disease virus (EHDV) or bluetongue virus (BTV) from an affected animal; and 4) observation of hunter-killed deer that showed sloughing hooves, oral ulcers, or scars on the rumen lining. These criteria, which have remained consistent during the entire 40 years, provide information on HD mortality and morbidity, as well as validation of these clinical observations through confirmatory laboratory efforts. In 1992, our SCWDS virology laboratory came online,

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allowing us to include additional validation and supporting information on the specific BTV and EHDV associated with these HD events. Since 1992, we have isolated more than 2,000 EHDV and BTV, some of which were not previously known to exist in the United States. In 1980, it is safe to say that none of us imagined that this survey would last for 40 years, nor could we envision the wealth of data that it would generate.

Survey results clearly define the distribution of HD in the United States, but the story provided by these data goes much further by advancing our understanding of this disease. There are distinct epidemiologic patterns over this range; for example, regional HD patterns can be associated with predominately acute and fatal disease, mild disease and recovery, or a more stable pattern of subclinical infections. These patterns can and have been explained through supportive research showing they are driven by population immunity and they are predictable. However, the distribution and epidemiology of HD has changed over time. Reports of HD have expanded throughout much of the northeastern and central United States over time, and these range expansions have been accompanied by an increasing occurrence of large-scale regional HD outbreaks. In addition, new serotypes of EHDV and BTV have been detected increasingly in the United States since 2000. These observations are troublesome, and it is unknown what the long-term effects of these changes will be.

In this and the next three issues of *SCWDS BRIEFS,* we will provide examples of how data provided by the HD survey continue to inform both research needs and management. We are going to start with a final update of our 2019 virologic data and, for perspective, visit retrospective survey data to visualize the long-term epidemiologic trends.

Part I: 2019 HD Season and a Closer Look at the Ohio River Valley

During 2019, 378 submissions were received from 25 states for HD testing. These included samples from 348 white-tailed deer, 13 elk, 10 mule deer, and four pronghorn antelope. Three cattle associated with an outbreak of EHDV-2 in West Virginia were also tested. The 25 states included Alabama, Alaska, Arkansas, Delaware, Florida, Georgia, Idaho, Indiana, Kansas, Kentucky, Louisiana, Maryland, Missouri, Mississippi, Montana, Nebraska, New Jersey, North Carolina, North Dakota, Pennsylvania, South Carolina, Tennessee, Virginia, Wisconsin, and West Virginia. A total of 209 virus detections from 18 of these states were made from white-tailed deer, mule deer, elk, and cattle; these included EHDV-1, EHDV-2, BTV-2, and BTV-13.

Much of the reported HD activity during 2019 was attributed to infections with EHDV-2, particularly in those states bordering the Ohio River including Indiana, Kentucky, Ohio, and West Virginia. When included with the retrospective survey data, the 2019 data support a major geographic expansion of HD during the last 40 years that is primarily associated with EHDV (Figure 1). In addition, the increasing frequency of large-scale outbreaks (2007, 2012, 2017, 2019) is clearly apparent (Figure 2).

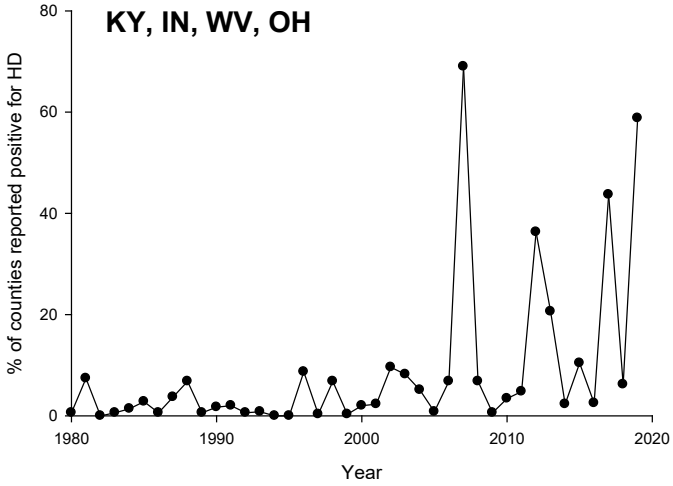

Figure 2: Percent of counties in Kentucky, Indiana, West Virginia, and Ohio where HD was reported by state fish and wildlife agencies as part of the SCWDS annual HD survey (1980-2019). A marked increase in the reporting of large-scale outbreaks since 2007 is apparent.

Although not shown, this trend is consistent within all four states, and in some years reports were received from most of the counties within the state. For example, in 2007 HD was reported from 69%, 71%, and 93% of the counties in Ohio, West Virginia, and Kentucky respectively; in 2019, HD was reported from 89% of counties in Indiana. Similar to 2019, all of the 2007, 2012, and 2017 outbreaks were associated with EHDV-2, but many other viruses including EHDV-1, EHDV-6, BTV-1, BTV-3, BTV-10, and BTV-17 have been documented from these states during this time. BTV-1 and BTV-3, which were isolated in West Virginia in 2018 and 2016, respectively, were considered exotic in North America before the 2000-2009 decade. At this time, we cannot definitively state the cause of these changes; however, northern range expansion of HD,

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increasing large-scale outbreaks, and the introduction and establishment of exotic BTV and EHDV serotypes may be associated with long-term changes in climate, including changes in wind, precipitation, and temperature patterns that affect vector population dynamics, vector competency, and dispersal. These relationships need to be explored.

We hope that all of you who have and continue to participate in the annual survey or submit samples for virologic testing find the data that we provide useful to management. We also want all of you to know how valuable your continued support is in providing a comprehensive data set to detect and understand these long-term HD patterns and trends. This dataset will hopefully facilitate a better understanding of the population, vector, and environmental drivers behind these changing patterns. A special thanks to West Virginia (Jim Crum), Kentucky (Chrissy Casey), Ohio (Mike Tonkovich), and Indiana (Nancy Boedeker) for providing 2019 HD survey data prior to our annual request. (Prepared by Dave Stallknecht, Natalie Stilwell, and Mark Ruder)

CWD Prions Detected in Deer Semen

A recent study published in *PLoS ONE* by prion researchers in the United States and Chile reports the detection of chronic wasting disease (CWD) prions in semen and reproductive tissues (i.e., testicle and epididymis) collected from naturally infected, captive white-tailed deer herds undergoing depopulation. A total of nine CWD-positive deer at either early stage (n=4) or late stage (n=5) of the incubation period (i.e., time from infection to clinical disease) and 12 negative control animals were included in the study. Test samples were concentrated to increase probability of detection and tested for the presence of CWD prions by protein misfolding cyclic amplification (PMCA), a highly sensitive test capable of detecting prions at much lower quantities than traditional diagnostic tests (e.g., enzyme linked immunosorbent assay [ELISA] and immunohistochemistry [IHC]). Overall, the PMCA test detected CWD prions in one or more samples from 6 of 9 deer, including semen (5/9), testicle (5/9), and epididymis (6/9). It is important to note that all of these bucks were in the pre-clinical stages of infection, meaning they were not yet exhibiting clinical signs of CWD. As with any good research finding, this study leaves us with more questions than answers. Paramount is whether or not the detection of CWD prions in these tissues equates to viable transmission pathways (e.g., sexual contact and artificial insemination) in the real world. Unfortunately, this will require experiments that may take years to complete, so clarity on this issue will not be immediately forthcoming.

Artificial insemination is a common practice in the captive cervid industry and semen is routinely shipped between facilities in different states. Whether this practice presents a significant risk for CWD introduction has not be definitively shown. Unfortunately, this can be added to the list of uncertainties surrounding transmission of CWD in nature. However, it is clear that CWD transmission can be efficient, and the consequences of CWD introduction and establishment in new areas are severe. Accordingly, agriculture and wildlife agencies are forced to make decisions to lower transmission risk based on our current scientific understanding, even when uncertainty exists. Efforts should continue to stop the movement of susceptible cervids (live or dead, whole or part) across the landscape in order to lower the risk for unnatural spread of CWD. The full text of this article can be accessed on-line at: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0226560 (Prepared by Mark Ruder)

Heterakis in Game Birds – A New Finding for an Old Parasite of Turkeys

Histomoniasis (i.e. blackhead) is a parasitic disease of wild and domestic gallinaceous birds caused by the parasite *Histomonas meleagridis*. The parasite is fragile and will die soon after being excreted in the feces. Although direct transmission has been reported between domestic turkeys under high bird densities, this is unlikely to occur among free-ranging birds. To counter this, *Histomonas meleagridis* uses an interesting transmission pathway in which it depends on another parasite, the cecal nematode, *Heterakis gallinarum*. The histomonads can enter the eggs of *Heterakis gallinarum* where they are protected until these worm eggs are ingested by another host.

Heterakis gallinarum infects a wide diversity of gallinaceous birds including turkeys, domestic chickens, pheasants, guinea fowl, grouse, chukars, quail, and prairie chickens, among others. Two other *Heterakis* species occur in wild birds in the US including *Heterakis isolonche* in pheasants, ruffed grouse, quail and others, and *Heterakis dispar* in waterfowl. In most species, *Heterakis* infections alone do not result in significant disease in the host;

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however, ring-necked pheasants with relatively high *Heterakis gallinarum* burdens can develop cecal lesions and clinical signs.

The susceptibility of gallinaceous birds to *Histomonas meleagridis* is highly variable and, in general, chickens, guinea fowl, and pheasants are more resistant to histomoniasis. These species experience mild to no clinical signs associated with *Histomonas meleagridis* infections and can excrete high numbers of heterakid eggs, making them potential reservoirs for the parasite. Other gallinaceous species, such as domestic and wild turkeys, ruffed grouse, northern bobwhite, and chukars, are more likely to develop severe lesions and experience moderate to severe disease associated with *Histomonas meleagridis* infection.

Although we generally know host ranges for these *Heterakis* species, we conducted a study to better understand the prevalence and host-preferences among wild and propagated game birds and poultry from Pennsylvania. We also genetically characterized the parasites to determine if there were any regional differences in the parasites. A total of 399 birds of eight species (wild turkey, domestic turkey, domestic chicken, chukar, ring-necked pheasant, ruffed grouse, American woodcock, and *Anas* spp. dabbling ducks) were examined for *Heterakis* spp. Five of these species were infected with *Heterakis* nematodes, including *Heterakis gallinarum* in captive-raised ring-necked pheasants (81% positive), free-ranging wild turkeys (60%), domestic chickens (48%), and chukars (10%) and *Heterakis isolonche* in ruffed grouse (81%). No *Heterakis* species were identified in the domestic turkey, woodcock, or dabbling ducks. These data show that captive propagated ring-necked pheasants and cage-free floor-raised chickens commonly harbor *Heterakis gallinarum* and could potentially contribute to disease transmission to susceptible host species.

Our genetic characterization provided an interesting finding. The parasites that were morphologically consistent with *Heterakis gallinarum* did not fall within the same genetic clade. Parasites from the chukar, ring-necked pheasants, and chickens were included in a large clade of *Heterakis gallinarum* from domestic chickens in several countries. However, the *Heterakis gallinarum* from the wild turkeys were in a distinct genetic clade that was more similar to *Heterakis isolonche* from the ruffed grouse. Currently it is unknown if this group of *Heterakis* worms from wild turkey represents a genetic variant of *Heterakis gallinarum*, a variant of *Heterakis isolonche*, or a novel species. This is particularly important because *Heterakis gallinarum* is a known vector for *Histomonas meleagridis*, but *Heterakis isolonche* is not currently considered a vector. Additional studies are needed to better understand the distribution of this clade of worms and understand the vectoral capacity of these worms for *Histomonas meleagridis*. SCWDS is interested in continuing this research, so please reach out if you are interested and capable of collecting *Heterakis* spp. from wild turkeys. A manuscript has been accepted in the journal *Avian Diseases*. (Prepared by Michael Yabsley, myabsley@uga.edu)

Aural Abscesses in an Eastern Box Turtle

An eastern box turtle (*Terrapene carolina*) with severe bilateral facial swelling (Figure 1) was discovered on a hiking trail in Athens, Georgia, in August of 2019. The prognosis of recovery was deemed poor due to the severity of the lesions and thus, the turtle was submitted to SCWDS for euthanasia and postmortem evaluation. On necropsy, the turtle was emaciated and had bilateral abscesses overlying both ears (i.e., aural canals; Figure 2). Microscopic evaluation and bacterial culture supported the diagnosis of bilateral aural abscesses in this eastern box turtle.

Aural abscesses are well documented in captive turtles, but this condition is also diagnosed frequently among wild box turtles that present to wildlife rehabilitation facilities. Aural abscesses typically develop in immunocompromised individuals from a bacterial infection in the middle ear. In captive turtles with poor nutrition, aural abscesses are often associated with vitamin A deficiency. Hypovitaminosis A is a predisposing factor for middle ear infections by inducing changes in the mucous-secreting epithelium lining the middle ear (e.g., squamous metaplasia, hyperplasia, and hyperkeratinization). These changes in captive turtles can cause sloughed epithelium to accumulate and form a dense plug with secondary bacterial infection in captive turtles. Numerous secondary bacterial invaders have been isolated from these abscesses in captive and free-ranging box turtles.

Aural abscesses have been documented to persist in some individual turtles for months to years without treatment. Although some turtles are seemingly unaffected by these abscesses, the

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more severely diseased individuals can exhibit behavioral changes, such as loss of appetite, increased heat-seeking behavior, prolonged periods spent soaking in water, and inability to close shell.

Figure 1. Eastern box turtle with bilateral aural abscesses.

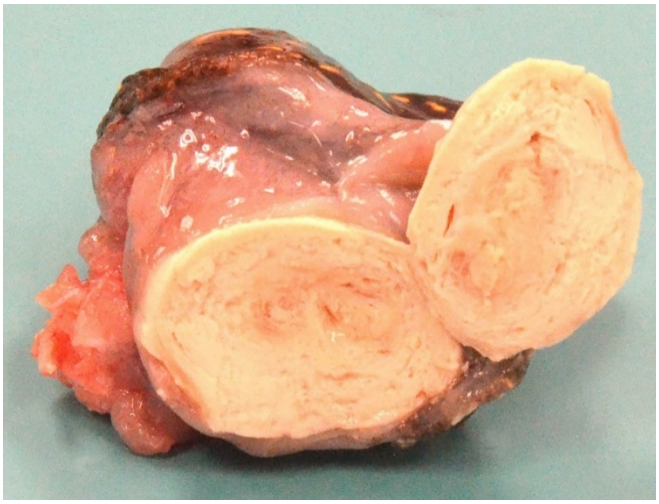

Figure 2. Cut surface of one of the abscesses in the middle ear.

While spontaneous regression of aural abscesses has been observed, treatment often is necessary. The only available treatment for aural abscesses is surgical removal of the firm abscess from the middle ear. This procedure requires general anesthesia and prolonged wound management, and therefore is not a viable option in every case. Although the procedure is thought to be effective, the long-term success in wild box turtles, a long-lived species, is not well-understood. Instances of reoccurrence of these abscesses following successful surgical treatment occasionally have been documented. However, the proportion of wild turtles that are treated and released that develop subsequent, recurring aural abscesses is currently not known. Aural abscesses are generally not thought to have population-level impacts in wild box turtles. Individuals who find such turtles should contact their state wildlife agency or a licensed wildlife rehabilitator.

Proposed causes for the development of aural abscesses in wild box turtles include chronic infections, malnutrition, and chronic exposure to environmental contaminants, such as organochlorine compounds. Recent research has focused on the effects of exposure to immuno-suppressive organochlorine pesticides, such as dichlorodiphenyltrichloroethane (DDT), in relation to aural abscesses in wild eastern box turtles, aquatic turtles, and sea turtles. Although use of DDT has been halted, it remains an environmental contaminant with bioaccumulative effects. Research has revealed a strong but complex link between turtles with aural abscesses and high hepatic organochlorine concentrations. Organochlorine (OC)-associated disturbances in vitamin A metabolism have previously been documented in several species, thus suggesting a possible environmental chemical-induced hypovitaminosis A cause of aural abscess formation in wild turtles. However, the underlying predisposing factors for development of aural abscesses in free-ranging box turtles ultimately remain unclear. Additional research is needed to fully understand the development of aural abscesses in wild turtles, including a better understanding of possible associations between organochlorine exposure and geographical variations with the development of aural abscesses in wild turtles. SCWDS thanks the Georgia Department of Natural Resources and Dr. John Maerz of the Warnell School of Forestry and Natural Resources, University of Georgia, for submission of this interesting case. (Prepared by Jennifer Starvetsky, Lincoln Memorial University, Melanie Kunkel, and Nicole Nemeth).

Feline Leukomyelopathy in Bobcats and Florida Panthers

In the spring and summer of 2018, the Florida Fish and Wildlife Conservation Commission (FWC) became aware of a neurologic syndrome in multiple, free-ranging bobcats and panthers within the Corkscrew Swamp in Collier County. Two Florida panther kittens and two young adult bobcats were observed via remote video to be ataxic with hind limb paresis (weakness) over multiple months. Clinical signs remained static after first observation, and mental status appeared normal. No other species in the area were known to be affected, and

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at least three other panthers (including the mother of the two kittens) and one bobcat in the area appeared normal. One bobcat was submitted to the SCWDS Diagnostic Services Section after death from natural causes in July of 2018, and a second was submitted in December of 2019.

Both bobcats were from south Florida (Collier County) and were in poor nutritional condition with moderate to severe muscle wasting, primarily in the hind limbs. The second had evidence of trauma, which was likely secondary to the neurologic condition. Numerous sections of spinal cord were microscopically examined, and severe lesions of symmetric white matter degeneration were evident throughout, specifically within motor neuron tracts.

Given that multiple felid species of different ages within a concentrated area have been affected, potential infectious, toxic, and nutritional causes have been considered. The symmetric and non-inflammatory nature of the lesions suggested a toxic or nutritional cause rather than infectious, and vigorous testing across all categories was conducted on the initial bobcat case. Toxic compounds, including desmethylbromethalin and organophosphates, were not detected. Heavy metal levels, including mercury, appeared to be within acceptable ranges for this species, and lead was not detected. Infectious agents were not detected (including testing for West Nile virus, eastern equine encephalitis virus, pseudorabies virus, mammalian bornavirus, rabies virus, and canine distemper virus). The vitamin A levels in the first bobcat with feline leukomyelopathy were significantly lower than those of an unaffected, control bobcat (200 ppm vs. 4000 ppm, respectively). Copper levels in the clinically affected bobcat also were subjectively low. These results raise the suspicion of vitamin A deficiency as a potential cause, as the microscopic lesions are similar to laboratory studies in cats fed vitamin A deficient diets. Also, vitamin A deficiency is a suspected cause of ataxic syndromes in captive lions and cheetahs. However, toxins remain an important consideration and efforts to assess for both micronutrients and toxicants in tissues of both affected and nonaffected bobcats continue. A combination of contributing factors may be at play.

Throughout 2019, the same neurologic syndrome has been documented in multiple Florida panther kittens and bobcats across a broader region of Florida as far north as Jacksonville. Public awareness of the syndrome recently has been heightened, and the FWC is working with the public and other partners to track the disease. Although more data are needed, observations by the FWC suggest that young felids are most susceptible.

Spinal cord degeneration severely impairs the affected cats' ability to walk, and therefore, the ability to evade danger and hunt. As a result, the affected cats are more prone to starvation, dehydration, or trauma. The cause of this syndrome is not known, therefore the potential implications on wildlife management and conservation remain unclear. However, there are valid concerns that this syndrome could negatively impact Florida panther conservation efforts. The FWC, SCWDS and other partners, will continue the epidemiologic and diagnostic investigation and primary objectives are to further characterize the disease, determine an underlying cause, and document the geographic distribution. Given the known geographic distribution of confirmed cases in Florida, SCWDS urges surrounding states to be vigilant and investigate reports. For suspect bobcat cases outside of Florida, please contact SCWDS to coordinate sample submission. Please also forward any suspicious video/photos or observation reports to SCWDS (Nicole Nemeth and Mark Ruder). Bobcats with differing severity of disease manifestation can be viewed at the following link: https://www.wyff4.com/article/wildlife-officials-are-investigating-why-panthers-are-seen-stumbling-and-falling-down-1566400248/28772843#
(Prepared by Martha Frances Dalton, Nicole Nemeth, and Alisia Weyna, SCWDS; Lara Cusack and Mark Cunningham, FWC).

Changing SCWDS Faces: Recent Arrivals

In our last issue of the *SCWDS BRIEFS*, we highlighted our staff and students who recently graduated or moved on to advance their careers. Here, we want to welcome our new students and staff who have arrived.

Ms. Erin Box was hired as a research technician in January 2020, but has previously served as a student assistant at SCWDS. She is also a Master of Science (MS) student in the College of Veterinary Medicine. She primarily works on the guinea worm eradication project and is investigating the roles of aquatic hosts as possible paratenic/transport hosts for *Dracunculus* spp.

Dr. Brian Dugovich joined SCWDS in June 2019, as a post-doctoral research associate after completing his PhD in Zoology at Oregon State University (OSU). In 2010, Brian received his DVM from OSU

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and worked as a clinical veterinarian for three years prior to his PhD program. His PhD research explored wild ungulate disease ecology and ecoimmunology. Brian serves SCWDS member agencies as a wildlife disease diagnostician and supports other service, research and training activities.

Dr. Raquel Francisco joined SCWDS in the fall of 2019 as a Master of Science (MS) student in the Warnell School of Forestry and Natural Resources. Dr. Francisco received her DVM from the University of Florida and worked as a clinical veterinarian in Oregon prior to her move to the University of Georgia. Her MS research will explore the antimicrobial resistance determinants carried by the European white stork along an anthropogenic gradient.

Ms. Kayla Guinn joined SCWDS in 2018 as a research technician after receiving a MS in Pre-Clinical Sciences from Mercer University. Kayla helps to manage a virology laboratory where she serves SCWDS member agencies by providing diagnostic support for the SCWDS Diagnostic Service, as well as a variety of other surveillance and research projects. In addition, Kayla is pursuing a PhD in the Comparative Biomedical Science program.

Dr. Melanie Kunkel joined SCWDS during the fall of 2018 to begin her PhD. Melanie received her DVM and Master of Public Health in 2017 from the University of Missouri and worked as a clinical veterinarian for a year before moving to Athens. Melanie's doctoral research centers on better understanding West Nile virus infection in game birds. In addition, Melanie serves SCWDS member agencies as a wildlife diagnostician on the SCWDS Diagnostic Service and is involved in other aspects of surveillance, research and training projects.

Dr. Rebecca Radisic joined SCWDS and the Department of Pathology in July 2019 to complete a residency in anatomic pathology with a wildlife emphasis. Rebecca received her DVM from the University of California-Davis School of Veterinary Medicine in 2019. Rebecca is serving SCWDS member agencies as a diagnostician on the SCWDS Diagnostic Service throughout her residency training.

Dr. Jorge Rojas recently joined SCWDS and the Warnell School of Forestry and Natural Resources, where he is pursuing a PhD. A native of Costa Rica, Dr. Rojas received his veterinary degree from the Universidad Nacional de Costa Rica and has been active in research and conservation of the Central American tapir. His PhD research will focus on the health and spatial ecology of the Baird's tapir in Northwest Costa Rica, where tapirs are at high risk for human-wildlife conflict.

Ms. Julia Seixas joined SCWDS and the Warnell School of Forestry and Natural Resources in 2019 to start her MS. Prior to her time at the University of Georgia, Julia received her bachelor of Science degree from Pennsylvania State University where she completed research on a serum-based pregnancy test in free-ranging elk. Her Master's research will center on better understanding the productivity of urban American white ibis that are breeding at urban rookeries in south Florida, and their nestling pathogen dynamics.

Dr. Natalie Stilwell joined SCWDS as a post-doctoral research associate in September 2019. Natalie completed her MS and PhD degrees in fisheries and aquatic animal health at the University of Florida after receiving her DVM from Auburn University in 2010. While at SCWDS, Natalie is heavily involved in our EHDV and BTV diagnostics, as well as research projects with our states involving hemorrhagic disease of deer, chronic wasting disease, and wild turkey health, among others.

Mr. Michael Tanner joined SCWDS as a research technician in October 2019. Michael received his MS in Medical Microbiology from the University of Georgia in 2005, and has extensive experience in laboratory techniques, previously working in both academic and industry laboratory settings. In a short amount of time at SCWDS, he has become a vital asset to the daily operations of a virology lab, currently focusing his efforts on assisting with the isolation and identification of avian influenza viruses.

Dr. Alisia Weyna arrived at SCWDS and the Department of Pathology in July 2019 to begin her residency training in anatomic pathology with a wildlife emphasis. Alisia received her DVM from the University of Wisconsin-Madison in 2019. In addition to serving SCWDS member agencies as a diagnostician on the SCWDS Diagnostic Service throughout her residency, she is also completing a MS degree focused on "green lung disease" in white-tailed deer in the Comparative Biomedical Sciences program.

We welcome our new arrivals to the SCWDS family and look forward to SCWDS supporters getting to know them. (Prepared by SCWDS Faculty)

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