SCWDS BRIEFS

A Quarterly Newsletter from the

Southeastern Cooperative Wildlife Disease Study
College of Veterinary Medicine
The University of Georgia
Athens, Georgia 30602

Phone (706) 542-1741 **FAX (706) 542-5865**

Volume 36 **October 2020** **Number 3**

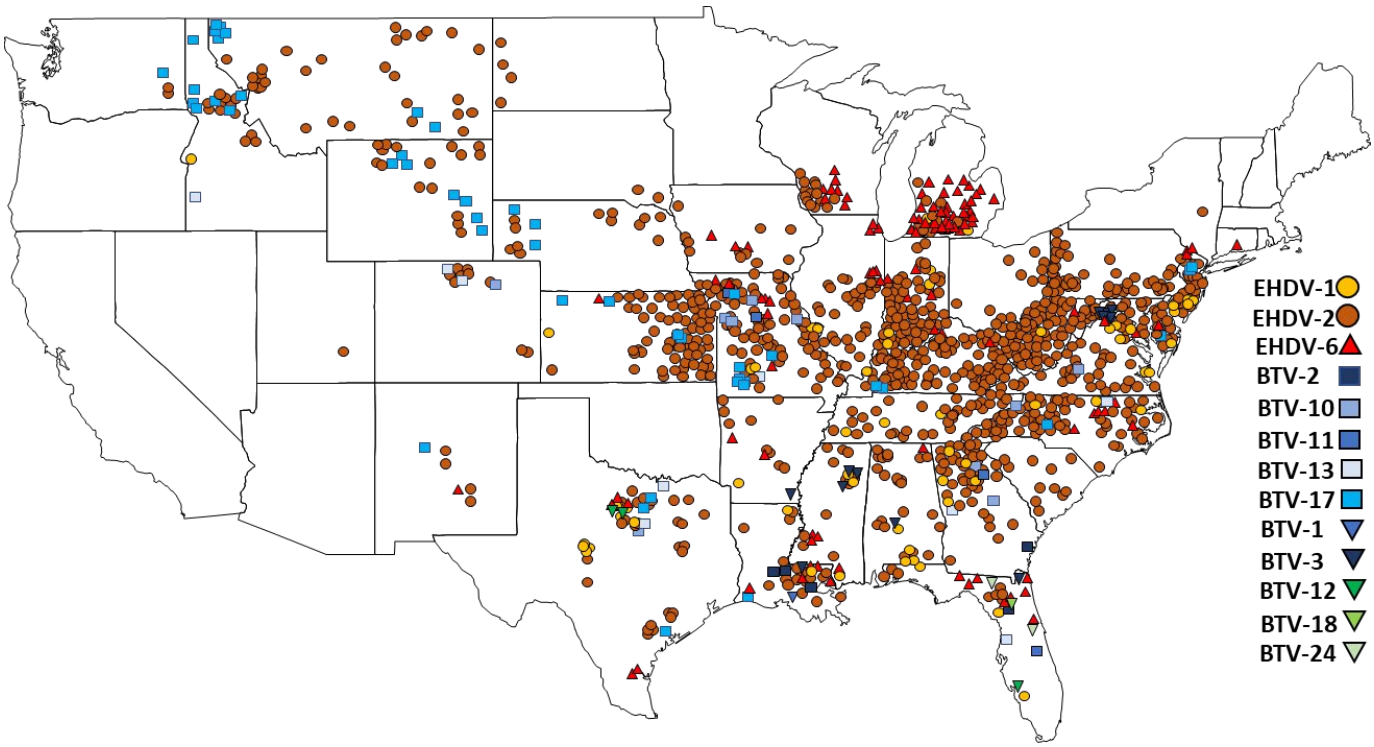

Figure 1. Distribution of EHDV and BTV isolations made by SCWDS 1991-2019.

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

In the last three issues of *SCWDS BRIEFS,* we provided examples of how data from a long-term, collaborative survey have been used to document the northern expansion of hemorrhagic disease (HD) in North America, how such data can provide a map of HD risk across broad landscapes, and how climatic and ecological factors can serve as drivers of HD outbreaks. The HD Survey data that feeds these studies are syndromic and based on reporting of suspected HD-related deer mortality. Therefore, a critical component of this surveillance is to confirm these HD reports through the detection of epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV). In part four of this series, we highlight how our efforts in the realm of diagnostic virology over the past 30 years have not only supported the HD Survey but also have provided a valuable and shared resource to better our understanding of HD.

Part 4: The value of diagnostic support

When the National Hemorrhagic Disease Survey was initiated in 1982, SCWDS had no in-house virology capabilities to confirm suspected HD cases. This changed in 1991 when EHDV-1 was isolated from a white-tailed deer from Lewis County, Tennessee. At that time, we were working in a lab that was made available to us by the Department of Pathology, and we performed virus isolation using embryonating chicken eggs.

Continued…

In 1999 we began developing our in-house capabilities. Since then, virus detection techniques have evolved from egg inoculation, to cell culture in combination with real-time, reverse transcriptase PCR detection and sequencing. These efforts have resulted in the isolation of more than 1200 EHD and BT viruses, and as shown in Figure 1, we have over time assisted and provided virologic data to 40 states. Individual state efforts related to EHDV and BTV diagnostics are not all represented on this map. Many states utilize our service only for specific needs, such as serotyping, and many others, especially SCWDS non-member states, utilize other diagnostic labs for testing. These data are not included in Figure 1, but are provided to us each year by all states as part of the National HD Survey.

The ability to identify specific viruses associated with an HD case or outbreak has immediate value to wildlife managers related to risk communication, as it is important to identify the cause of any unexplained mortality event. EHDV and BTV are not the only pathogens associated with potentially fatal disease in white-tailed deer, and this list includes several important foreign animal diseases (FAD). The availability of virologic data also supports situational awareness of mortality events, enabling fish and wildlife agencies to provide the public with reliable information related to disease risks and expected outcomes. The value of the virologic data and the EHDV and BTV isolates that we obtain does not end with these immediate benefits, as highlighted in this series of *SCWDS BRIEFS* articles.

The viruses that we have obtained also have been used extensively in both applied and basic research to greatly advance our understanding of the epidemiology and changing nature of HD; both of these are complicated due to the multiple EHDV and BTV serotypes that can result in disease. From the virologic data we have accumulated, there are several consistent relationships that if understood, may better inform our knowledge of HD epidemiology. For example, most BTV isolations have come from submissions from the western states and from coastal plain and piedmont physiographic regions in the Southeast. All recent, large-scale regional HD outbreaks have been dominated by EHDV-2 and this has been the most prevalent serotype during all years, especially in the eastern United States. Changing patterns of serotype diversity also are apparent. Since 2004, when we isolated BTV-1 from a white-tailed deer in Louisiana, with the assistance of the National Veterinary Services Laboratory, we have detected a total of five exotic BTV (BTV-1, -3, -12, -18, -24) and one exotic EHDV (EHDV-6). Since 2006 when first detected, EHDV-6 has become established in the United States and in some areas (e.g., Wisconsin and Michigan), appears as frequently as EHDV-2. Likewise, BTV-3 has extended its range and may have become established in the United States; in 2016, this virus caused a localized HD outbreak in Virginia and West Virginia. EHD and BT viruses isolated by SCWDS have been shared with both national and international collaborators to validate diagnostic assays and better understand phylogenetics of these viruses. They have been utilized in experimental studies to research pathogenesis and the immune response, to understand the potential for reassortment, and to determine vector competence and the effects of temperature on viral replication in these vectors. Many of these studies were conducted by former SCWDS graduate students, and this work was made possible by the availability of these relevant and contemporary field viruses.

It is both fact and cliché that research only leads to more questions, and the virologic data have provided a wealth of such questions. For example, why is EHDV-2 the predominant serotype in the eastern United States while BTV and EHDV both are common in HD cases in the western states? This difference could be related to differences in vector communities, host populations, or both. Why has EHDV-2 been associated with all recent regional outbreaks; is this serotype more fit in vector or host species? Why is EHDV-6 commonly associated with HD mortality in northern areas? Is this a result of reassortment, vector/host fitness, low herd immunity, or all of these? What allows exotic viruses like EHDV-6 and BTV-3 to establish in a new ecosystem? What specific adaptations to hosts or vectors are needed for this to happen and to what extent does reassortment facilitate these adaptations? What will the effects of a changing climate have on HD epidemiology? Will subtype diversity patterns change; will exotic serotypes of BTV and EHDV continue to be detected, and if so, will they result in changes in HD epidemiology that will alter regional risks?

Continued…

After 40 years of the National HD Survey and 30 years of gathering virologic data supporting this survey we have learned a great deal, but as the questions above indicate, the many remaining unknowns highlight the potential for unexpected changes in the epidemiology of HD. With that said, the value of this survey has not diminished with time as it continues to provide a solid foundation to move our understanding of this disease forward. The team of wildlife professionals that have contributed to the generation of the data to make this work possible cannot be thanked enough. Likewise, the SCWDS team that has isolated and identified these viruses, reported results, and conducted much of the research mentioned in this article were and are critical to these efforts. We thank all of you and hope the work will continue. (Prepared by Dave Stallknecht)

H10 Influenza Viruses in Ruddy Turnstones: Source or Sink?

We have been collaborating with scientists at St. Jude Children's Research Hospital since 1999 to better understand the epidemiology of influenza A virus (IAV) in shorebirds at Delaware Bay, New Jersey. This field site has been monitored by St. Jude since 1985, creating one of the longest continuous IAV data sets in the world! Thousands of IAVs have been isolated, characterized, and used to not only understand influenza ecology at Delaware Bay, but also to provide viruses used for empirical IAV research. Despite these many years of research, facets of IAV ecology and epidemiology remain unexplained. We currently do not understand why IAV prevalence is consistently highest in Ruddy turnstones (RUTUs, *Arenaria interpres*) compared to other shorebirds of the order Charadriiformes. Subtypes isolated from RUTU at Delaware Bay seem to come and go between years with some hemagglutinin (HA) subtypes over- and under-represented both within and between years. Neither the extensive IAV subtype diversity observed annually in RUTU at Delaware Bay, nor the variation in this diversity between years can be explained. Subtype H10 viruses are frequently isolated at Delaware Bay and in many years represent the predominant subtype. This pattern of periodic appearance and predominance in combination with the large number of H10 isolations offered an opportunity not only to explore the genetic variation within North American H10 viruses, but also to determine if specific genetic lineages of these viruses are maintained in RUTU populations or are periodically replaced.

Delaware Bay habitats are utilized as refueling sites by RUTU during spring migration where they capitalize on the coincident horseshoe crab spawn. From Delaware Bay, RUTU proceed to the Arctic, where they form pairs and disperse for breeding. We hypothesized that this annual dispersal of birds (and resulting lower densities) would greatly reduce the likelihood of IAV transmission during the breeding season, possibly preventing the IAVs detected at Delaware Bay during spring migration from persisting in the population. If this is true, RUTUs and other shorebirds may serve as "sinks" for IAV genetic diversity, rather than contributing to the maintenance of IAVs that are specific to Charadriiformes.

To explore this research question, we focused on fully sequenced genomes from H10 IAVs from North and South American wild birds that are available in the Influenza Research Database (IRD; www.fludb.org). The resulting dataset included 914 isolates from 1953 – 2019. Of these, 365 (39.9%) were identical to at least one other isolate. Half of the isolates were from 28 identified species of the order Anseriformes (n=450; ducks and geese) and the other half were from 10 species of gulls, shorebirds, or terns from the order Charadriiformes (n=454). Genetic analysis to visualize the relatedness of each HA sequence to all others indicated that sequences from Charadriiformes were most like sequences from the same species and the same year. There were large clusters of very similar HA sequences from RUTUs and gulls at the same sampling site in a particular year. In these clusters, there were several instances in which the most closely related virus to each RUTU cluster was identified as a duck virus from a previous year. This pattern can be attributed to one of two scenarios. Either the H10 virus is introduced from a single source and then is transmitted within the RUTU population, or only permissible viruses with a narrow range of characteristics can replicate efficiently in RUTUs. Regardless of which mechanism or combination thereof permits infection of RUTUs with H10 IAVs, it is clear that individual H10 lineages found in these clusters of

Continued…

closely related RUTU viruses do not reappear in a subsequent year.

These data support our hypothesis that shorebirds, particularly RUTUs, are sinks for H10 IAV diversity. It is likely that the viruses annually detected in shorebirds at Delaware Bay originate from waterfowl. We have identified amino acid residues that differ between duck and shorebird viruses, indicating different selection pressures between these hosts. This may imply that IAVs require some host adaptation to be efficiently transmitted in RUTU. However, it appears that the longevity of a specific genetic lineage of H10 viruses within the RUTU population is short-lived and constrained by the biology and behavior of this species. (Prepared by JoJo Crum and Becky Poulson)

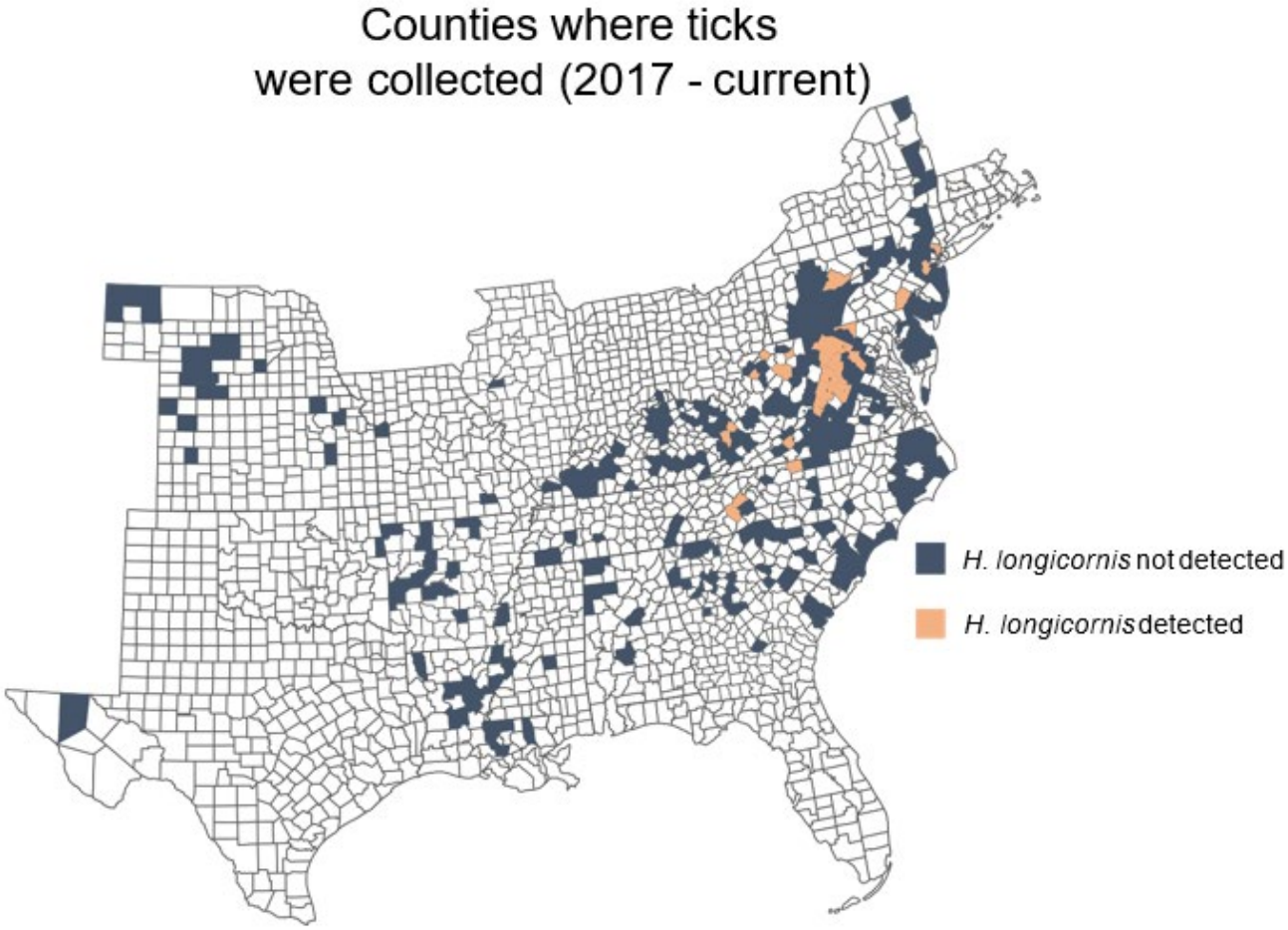

Figure 1. Map of states and counties sampled for *Haemaphysalis longicornis* using passive and active surveillance techniques. *Haemaphysalis longicornis* was detected in 29 counties from 7 states.

Surveillance for the Asian Longhorned Tick

Since its discovery in the United States in 2017, the exotic Asian longhorned tick (*Haemaphysalis longicornis*) has been found in 14 states on a variety of domestic animal and wildlife hosts. SCWDS in collaboration with partner agencies and member states has played a significant role in adding new state, county, and host records to the rapidly growing body of knowledge on *H. longicornis* in the United States. This has been done largely through a passive tick surveillance program working with state and federal wildlife agencies and wildlife rehabilitation centers distributed throughout parts of the eastern and southeastern United States. Ticks collected from wildlife hosts are submitted to SCWDS for morphologic identification and any *H. longicornis* suspects are confirmed through a secondary confirmation at the National Veterinary Services Laboratories (NVSL) and by using molecular techniques. Since beginning this surveillance, 1,435 samples have been submitted from 302 counties across 22 states. *H. longicornis* was detected in 29 counties in seven states (KY, MD,

Continued…

NC, NJ, PA, VA, WV) (Figure 1). The vertebrate host range was extensive and included 10 mammalian hosts (black bear, coyote, domestic dog, eastern cottontail, elk, gray fox, red fox, white-tailed deer, and woodchuck) and 3 avian species (brown booby, great-horned owl, and red-tailed hawk). It is noteworthy that all birds were found debilitated on the ground and were presented to wildlife rehabilitation facilities. In addition to providing key data about *H. longicornis*, this tick surveillance program provides valuable information regarding the host associations and distributions of other ticks native to the United States and relevant to wildlife, domestic animal, and human health. This work is ongoing, and we plan to continue these surveillance approaches involving wildlife agencies and wildlife rehabilitation facilities. To submit ticks or to request tick collection kits, please contact Dr. Michael Yabsley at myabsley@uga.edu.

Ticks collected from this study have also fueled many other research efforts both within SCWDS and with other collaborators. Notably, a recent study by Egizi et al. 2020 published in *Zoonoses and Public Health* (https://doi.org/10.1111/zph.12743) investigated the population genetics and origin of *H. longicornis* in the United States and utilized many of the ticks collected from our surveillance program. Results from this study suggest that *H. longicornis* was introduced to the United States on at least three occasions, likely from an area in Southeast Asia. In addition, we are collaborating with those same scientists to analyze the population genetics of *H. longicornis* from both the United States and from native regions using a different technique to further our understanding of the introduction of this tick. We welcome samples of *H. longicornis* from across its recognized range in the United States but have a special interest in increasing our representation from Pennsylvania and Tennessee. Please contact us to submit samples for this study.

We have also conducted active surveillance for ticks on wildlife and in the environment at sites in New Jersey and Virginia where known *H. longicornis* infections had been documented. These efforts have resulted in detections of *H. longicornis* on seven different wildlife species including raccoon, Virginia opossum, red fox, woodchuck, eastern cottontail, striped skunk, and white-tailed deer. We found certain host species (raccoons, Virginia opossums, and white-tailed deer) to be more likely to have *H. longicornis* infestations. These data combined with those from our passive surveillance suggest that in general, wildlife are important hosts for maintaining *H. longicornis* in the United States. More information on the active surveillance work was recently published in the journal *Transboundary and Emerging Diseases* (https://doi.org/10.1111/tbed.13722).

Figure 2. Photo of a questing Asian longhorned tick at a study site in Virginia.

Finally, both native ticks and exotic *H. longicornis* collected from the environment in Virginia (Figure 2) have been screened for various pathogens including an exotic parasite, *Theileria orientalis* Ikeda strain, which has been associated with cattle mortality in Virginia. To date, no *T. orientalis* have been detected in native tick species, but about 13% of *H. longicornis* collected from this site were positive for this important cattle pathogen. More details are available in a recent publication in *Ticks & Tick-borne Diseases* (https://doi.org/10.1016/j.ttbdis.2020.101450). This marks the first detection of a pathogen in host-seeking *H. longicornis* in the United States. While our surveillance further implicates *H. longicornis* as a vector for *T. orientalis* Ikeda, additional experimental transmission studies must be conducted to confirm this association and the tick's status as a vector. (Prepared by Alec Thompson and Michael Yabsley)

Leukocytozoonosis in a Wild Turkey

In August 2020, a wild turkey was found dead in the yard of a private citizen in Jackson County, North Carolina. The carcass was collected and submitted to the SCWDS Diagnostic Service by the North Carolina Wildlife Resources

Continued…

Commission (NCWRC). Gross examination revealed severely depleted fat stores and skeletal muscle atrophy. Dozens of yellow, semi-firm, proliferative, skin nodules were scattered across the head, along the neck, and at the medial canthus of the right eye. The spleen was markedly enlarged and mottled pale tan to gray, with numerous, small, flat, white foci across the capsular surface (Figure 1). Similar foci also were found on the liver and along the outer wall of the small intestine. Microscopic evaluation revealed moderate numbers of distorted blood cells in multiple organs (e.g., spleen, liver, heart, lung, bone marrow, kidneys, pancreas, and brain). Affected cells contained a clear, ovoid, 1 to 3-micron diameter, intracellular blood cell parasite (i.e., hemoparasite) that peripheralized the cell nucleus to one or both sides of the cell (Figure 2; arrows demonstrate affected blood cells). Other lesions attributed to these parasites included a dead (necrotic) area of liver, likely resulting from decreased blood supply due to blockage of nearby blood vessels with deformed, parasitized blood cells.

The gross and histopathologic changes in this turkey are characteristic of leucocytozoonosis, a disease caused by a protozoan hemoparasite (*Leucocytozoon* sp.) that infects both red and white blood cells. *L. smithi* is associated with wild and domestic turkeys, but other *Leucocytozoon* species can infect a variety of bird species. The parasite is primarily transmitted by black flies and less commonly by biting midges. Most infected birds show no clinical signs, but death can occur when high numbers of blood cells are infected. This can cause difficulty breathing, lethargy, and loss of appetite due to low red blood cell numbers (anemia), and formation of blood clots that block blood vessels, as was seen in this case. Additionally, organs that filter and process the blood (e.g., spleen and liver) may be enlarged, and low blood cell counts and deformed blood cells can lead to changes in blood flow and fluid leakage out of blood vessels (such as into the heart sac). Other life stages of the parasite can be found within pale nodules in the heart, liver, lung, and spleen, which are comprised of inflammatory cells. The skin lesions in this turkey were consistent with avian pox, and the turkey also tested positive for reticuloendotheliosis virus (REV). Pox is the most common infectious disease diagnosed in wild turkeys at SCWDS, and REV is similar to lymphoproliferative disease virus (LPDV) in its ability to infect and cause tumors in wild as well as domestic turkeys. These co-infections may have weakened the turkey's immune status and predisposed it to severe leucocytozoonosis.

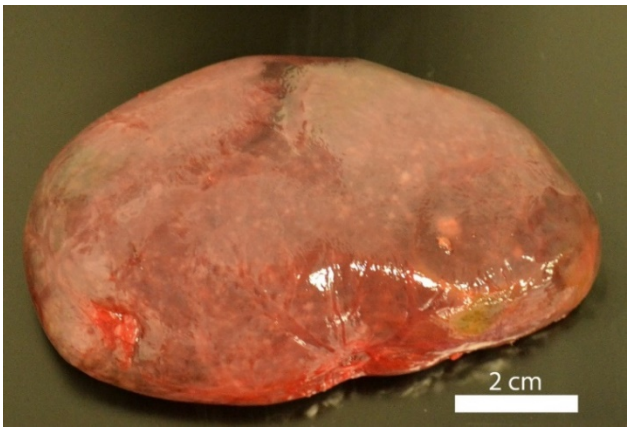

Figure 1. Markedly enlarged spleen from a wild turkey infected with *Leucocytozoon* sp. Note the multifocal, pale tan foci on the capsular surface.

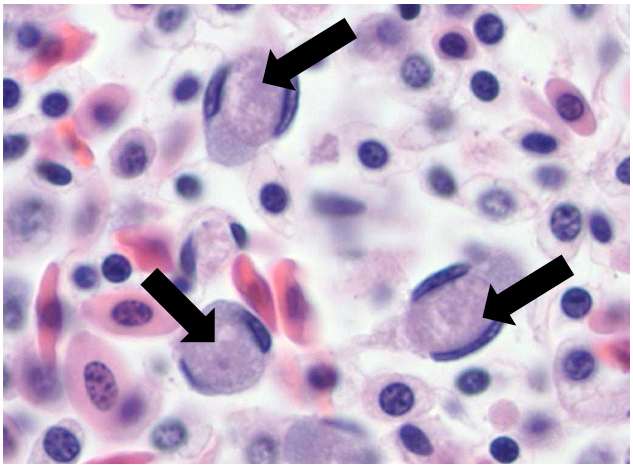

Figure 2. Photomicrograph of a blood vessel lumen in the liver of a wild turkey infected with *Leucocytozoon* sp. Clear vacuoles (gametocytes) indicated by arrows peripheralize red blood cell nuclei.

Many native North American bird species, as well as domestic poultry and captive exotic birds, can serve as intermediate hosts of *Leucocytozoon*. Among wild birds, ducks and geese are most severely affected, but wild turkey poults also may experience high mortality. However, infections with the parasite are more often detected in ground nesting birds, large-bodied birds, and raptors. Black flies or midges are the definitive hosts. Parasite transmission occurs when an infected fly bites a bird, followed by parasite invasion and maturation in the bird's liver over a 4-5 day period, eventually leading to development into the form (gametocytes) that

Continued…

infects blood cells. Gametocytes can then be ingested by another fly, perpetuating the cycle. Parasite accumulation within the blood (parasitemia) of birds occurs soon after infection and peaks in late April and early May to coincide with increased activity of arthropod vectors. During spring, waterfowl breeding grounds may be common sites for outbreaks with high mortality. Chronic leucocytozoonosis may be observed in birds year-round. These birds often recover and serve as a reservoir of infection for younger, more susceptible birds. *Leucocytozoon* spp. are more abundant in natural forested areas and cooler regions, and some studies show that the hemoparasite is more prevalent at higher altitudes. The best method of disease prevention is to control fly vectors. Clinically ill animals are not suitable for human consumption, although *Leucocytozoon* spp. are not known to infect humans.

We are grateful to James Tomberlin and the NCWRC for submission of this interesting case. (Prepared by Ava Vasyliev of Ohio State University, Alisia Weyna and Nicole Nemeth)

Changing SCWDS Faces: Recent Arrivals

The SCWDS family tree, with branches all over the world, continues to change and grow. Over the last year, several new students and staff have joined SCWDS.

Dr. Caitlin Burrell arrived in October 2020 as the new Staff Pathologist for the SCWDS Diagnostic Service. She brings a wealth of diverse training, experience and knowledge to SCWDS. Caitlin recently completed an MSc and a Zoological Pathology Residency at the University of Illinois. Her MSc research explored the variable susceptibility of coyotes, raccoons, and striped skunks to canine distemper virus. Caitlin received her DVM from Kansas State University in 2012, followed by a Small Animal Medicine Internship in Massachusetts in 2013, and a Zoological Medicine Internship at Texas A&M University in 2014. Following her internships, Caitlin completed a Veterinary Post-doctoral Fellowship at the Smithsonian Conservation Biology Institute where she worked extensively on giant and red panda conservation. Caitlin will play a major role in our SCWDS Diagnostic Service and will integrate into many other teaching, research and service activities.

Ms. Emily Doub joined SCWDS as a Research Technician in January 2020. A native Tennessean, Emily received her BS in Wildlife and Fisheries Science from the University of Tennessee in 2019. Prior to joining SCWDS she worked as a field technician on wildlife projects for Kansas Department of Wildlife, Parks and Tourism, as well as the United States Forest Service in Bridger-Teton National Forest in Wyoming. Emily is heavily involved with our USDA-funded arthropod surveillance project and works on numerous field and laboratory projects to understand the ecology of vector populations, such as *Culicoides* biting midges and numerous native and exotic tick species.

Mr. Ryan Grunert joined SCWDS as a Master's student (Comparative Biomedical Sciences in the College of Veterinary Medicine) in May 2020, but he has been working with us on various research projects for well over a year. He obtained his BS in Wildlife Sciences from the Warnell School of Forestry and Natural Resources at UGA and as an undergraduate student, was involved with several *Dracunculus* (Guinea worm) projects at SCWDS. For his MS research, Ryan will be continuing our long-term studies on mange in black bears. He will tackle genetic questions related to the host and the mite to better understand the epidemiology of this emerging problem in bear populations in the eastern United States.

Ms. Emma Kring joined SCWDS and the UGA Deer Lab at the Warnell School of Forestry and Natural Resources in August 2020 to begin her MS. Originally from Pennsylvania, Emma received her BS in Environmental Science from Juniata College in 2015, and comes to SCWDS with a wealth of wildlife experience. Most recently, Emma served as a Conservation Technician for Nebraska Game and Parks Commission. Prior to that, she worked on field research crews for mule deer (Nebraska and Wyoming), white-tailed deer (Pennsylvania), and elk (Missouri) projects. Emma's MS research will explore patterns of reported hemorrhagic disease in multiple Great Plains states (OK, KS, NE, SD, ND) over the past 40 years and investigate potential ecological and climatic variables that may underlie these patterns.

We welcome our new arrivals to the SCWDS family and look forward to SCWDS supporters getting to know them.

SCWDS BRIEFS

Southeastern Cooperative Wildlife Disease Study
College of Veterinary Medicine
The University of Georgia
Athens, Georgia 30602-4393

Nonprofit Organization
U.S. Postage
PAID
Athens, Georgia
Permit No. 11

RETURN SERVICE REQUESTED

Information presented in this newsletter is not intended for citation as scientific literature. Please contact the Southeastern Cooperative Wildlife Disease Study if citable information is needed.

Information on SCWDS and recent back issues of the *SCWDS BRIEFS* can be accessed on the internet at https://vet.uga.edu/scwds. If you prefer to read the BRIEFS online, just send an email to Jeanenne Brewton (brewton@uga.edu) or Michael Yabsley (myabsley@uga.edu) and you will be informed each quarter when the latest issue is available.