

SCWDS BRIEFS

A Quarterly Newsletter from the
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Volume 38

April 2022

Number 1

HPAI in North America – A Growing Wildlife Health Concern

In the February 2022 issue of the *SCWDS BRIEFS*, we provided a timely update on the highly pathogenic (HP) H5 influenza A virus (IAV) situation that was unfolding in North America. We also highlighted the potential threat of the 2.3.4.4 lineage of A/Goose Guangdong HP H5 IAV to wildlife health and provided a number of unresolved questions to highlight gaps in our knowledge of this pathogen, the clinical disease it can cause, and its potential role in North America, where IAV transmission historically is dominated by low pathogenic (LP) IAV. Unfortunately, the situation has drastically changed during the past few months, and since first reported by the Canadian Food Inspection Agency on December 22, 2021, 2.3.4.4B HP H5N1 IAV (hereafter, H5 HPAIV) has rapidly spread throughout most of North America, in both wild birds and poultry.

Wild avian populations in the Southeast have not been spared, and this ongoing outbreak is increasingly concerning to wildlife health in North America (Figure 1).



Figure 1. Photo from Volusia County, Florida, at site of black vulture H5 HPAIV mortality event sustained in cycle of vulture-to-vulture transmission through scavenging. Photo by Mark Cunningham.

As of the end of April, SCWDS had received 263 wild bird submissions from over 60 species and 13 states for testing. From these samples, H5 HPAIV was detected (by rrt-PCR at SCWDS) and confirmed (at NVSL) in 72 birds from Florida, Georgia, Kansas, Kentucky, North Carolina, South Carolina, and Virginia. Most samples that tested positive were from raptors (33/72, 46%) and waterfowl (28/72, 39%; Figure 2).

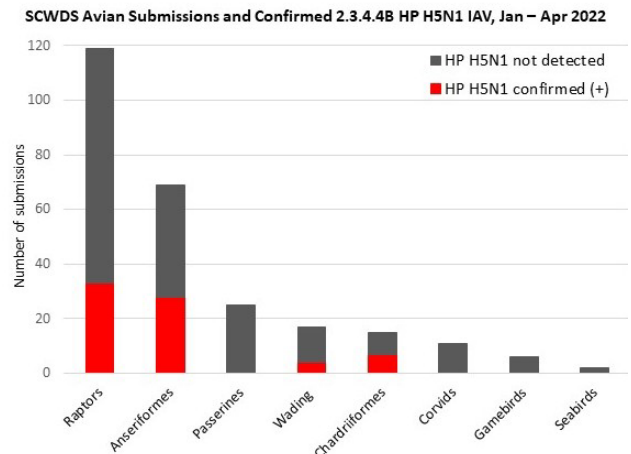


Figure 2. Bar graph showing number of bird submissions to SCWDS January-April 2022, and the number of birds in each group with confirmed 2.3.4.4B HP H5N1 IAV (red).

Through strong collaborations between SCWDS and our supporting agencies, we have learned a great deal about H5 HPAIV-related morbidity and mortality events by coupling pathology/laboratory-based results with real-time monitoring and field observations. Here we will highlight three specific examples.

Bald Eagles in the Southeastern United States: In February – March 2022, acutely fatal and systemic H5 HPAIV infections were confirmed in 22 bald eagles (BAEA, nestlings and adults) from coastal areas of FL, GA, NC and SC. Aerial BAEA nest surveys of coastal nests conducted by the Georgia Department of Natural Resources during March revealed 47% nest success – a sharp decline from

the 78% average (2015 – 2021). Further, the number of young fledged per occupied territory decreased during 2022, compared with fledge success from 2015 – 2021. These findings may indicate a negative impact on local BAEA populations through both direct mortality, and decreased recruitment. More recently, the number of H5 HPAIV positive BAEA cases has decreased but we do not know what the future may hold. From temporal and spatial perspectives, it appears that eagle mortality correlates with reported waterfowl infections and mortality. However, there are multiple instances of H5 HPAIV mortality in bald eagles occurring in locations in the absence of known waterfowl mortality, indicating the unfortunate sensitivity of predatory/scavenging raptors as indicators of H5 HPAIV circulation. Because of the local impacts we observed this year, vigilance and monitoring of eagle populations and detection of HPAIV are critical to better understanding this ongoing outbreak and to perhaps better understand future risks.

Lesser Scaup, Florida: In early February, increasing numbers of dead or neurologic lesser scaup (LESC) were reported on the East and Gulf Coasts of FL. Mortality estimates were in the thousands, with the last recorded LESC mortality at the end of March. There was no indication that other waterfowl species in the immediate area were affected. In the Southeast, this was the only large-scale mortality event detected in waterfowl despite the fact that H5 HPAIV was detected in numerous waterfowl species and populations in many southeastern states. Why this occurred in Florida is unclear and highlights the fact that we do not currently understand what factors place a specific species or population at risk or if such risk factors will vary between seasons or locations. For this reason, it is important to fully investigate these individual outbreaks beyond simply opportunistically surveying for H5 HPAIV.

Black Vultures, Florida: As LESC mortalities declined, black vulture (BLVU) mortalities increased rapidly in both case number and geographic extent with hundreds of documented mortalities suspected due to H5 HPAIV. Over 300 such mortalities were documented at one roost site in Volusia County alone, where BLVUs were observed scavenging fresh vulture carcasses (Figure 1). It is likely that initial infections in BLVUs originated through scavenging on infectious LESCs carcasses or those of other species. However,

once in BLVU, it appears that this virus is maintained at roost sites in a cycle of mortality-scavenging-mortality. More recently, significant BLVU mortality associated with H5 HPAIV has been confirmed in Maryland and South Carolina. This is a unique situation and although hooded vulture mortality associated with HP H5AIV has been reported in Africa, the magnitude of this outbreak is unprecedented. Likewise, this unique maintenance cycle that is independent of a waterfowl reservoir may be difficult to break.

As always, SCWDS remains committed to assisting member states and regional partners in disease investigations. The movement of waterfowl towards northern breeding grounds in late spring has seemingly reduced the transmission cycle across much of the Southeast, as reports of H5 HPAIV in wild birds in other parts of the United States and Canada continue at a quick pace. Whether this virus becomes endemic in North America remains unknown, but the current situation closely mirrors that experienced in Europe for the past few years, in terms of the diversity of species affected, geographic scope, mortality events reported, intensity of infection, and spillover into poultry. As we move through the summer months and think ahead to autumn southward migration of waterfowl and other avian species, please communicate with us about your plans for more targeted IAV surveillance (e.g., swab-based live, serology-live, or dead bird sampling) and specific needs you might have.

For more information:

- <https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/avian/avian-influenza/2022-hpai>
- https://www.fao.org/ag/againfo/programmes/en/empres/Global_AIV_Zoonotic_Update/situation_update.html

(Prepared by Becky Poulson, Mark Ruder, and Dave Stallknecht)

Highly Pathogenic Avian Influenza in Snow Geese

Approximately 30 snow geese (SNGO) were found dead or dying near a reservoir in Mitchell County, Kansas on March 4, 2022. Additional geese were lethargic, and exhibited neurologic signs such as shaking their heads, swimming in circles, and

difficulty walking straight. Numerous nearby reservoirs reported mortalities between 200 – 2,200 SNGO and multiple bald eagles, with some SNGO exhibiting similar neurologic signs. Kansas Department of Wildlife and Parks personnel submitted two geese carcasses from this outbreak for diagnostic evaluation.

On necropsy, one goose had disseminated dark red to light brown, flat foci dispersed throughout the pancreas and an enlarged spleen that was mottled dark red to purple. The other goose displayed similar multifocal to locally extensive red areas on the pancreas (Figure 1), as well as small hemorrhages scattered on the surface of the heart and hemorrhage covering the brain.



Figure 1. Multiple, widely scattered, dark red foci in the pancreas of a snow goose representing areas of acute cell death due to highly pathogenic avian influenza virus infection.

Microscopically, pancreatic lesions were associated with degeneration and cell death (necrosis). Multifocal to coalescing areas of necrosis accounted for the grossly mottled appearance of the spleen. In addition, over 80% of the liver from one goose was effaced by necrosis. Nearly all organs had vascular congestion. Despite clinical neurologic signs, there were no significant lesions in the brain of either goose. Clade 2.3.4.4B highly pathogenic (HP) H5 influenza A virus (hereafter H5 HPAIV) was detected in pooled oral and cloacal swabs from both geese by real-time reverse transcriptase polymerase chain reaction at SCWDS and confirmed by National Veterinary Services Laboratories. These SNGO were in excellent nutritional condition, consistent with rapidly fatal infection, and had no evidence of co-infections or other abnormalities.

Numerous species of birds, especially those within the orders Anseriformes (geese, swan, ducks) and

Charadriiformes (shorebirds, gulls), are natural reservoirs for all subtypes of IAVs. Infection prevalence varies by affected species, viral strain, geography, and season. Shorebirds have high rates of infection during spring migration staging on the Atlantic coast of North America. In addition, raptors and other scavenging birds (e.g., vultures, crows) can become infected after ingestion of infected waterfowl and other birds.

Since the first United States detection of H5 HPAIV by USDA-APHIS-Wildlife Services in January 2022, the virus has been detected in all North American flyways in numerous wild bird species. To date, SCWDS has detected H5 HPAIV in 20 avian species representing six Orders (i.e., Anseriformes, Falconiformes, Gaviiformes, Charadriiformes, Pelecaniformes, Ciconiiformes). The differences in disease severity between the geese in the present case versus some of the other avian species is not currently understood but may reflect species-related differences in susceptibility or population immunity. (Prepared by Chloe Goodwin and Nicole Nemeth)

Interpretation of EHDV and BTV Diagnostic Results

Each year SCWDS tests hundreds of white-tailed deer and other wild ungulates for epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV). In 2020 and 2021, our testing strategy was to utilize individual real-time reverse transcription polymerase chain reaction (rrt-PCR) tests specific for EHDV and BTV to initially screen sample submissions for the presence of each virus. When initial screens were positive, virus isolation (VI) on cattle pulmonary artery endothelial (CPAE) cells was performed to detect the presence of infectious EHDV or BTV (i.e., confirmation of initial screening). Samples (e.g., spleen, lung, or pooled combinations) originated from white-tailed deer that were submitted from more than 30 states. The data generated gave us the opportunity to compare results from rrt-PCR (detection of EHDV or BTV RNA) to VI (detection of infectious EHDV or BTV). As is often the case with wildlife sample submissions, varying degrees of sample quality were encountered and this was not considered as part of this evaluation.

For EHDV, 251 samples that tested positive by rrt-PCR at a cycle-threshold (Ct)-value below 40 that were then tested by VI. All samples with a Ct-value

above 40 were considered negative. With rrt-PCR, Ct-values decrease with increasing viral RNA load. As expected, the ability to isolate EHDV increased with decreasing Ct-value. At Ct-value ranges of <16, 16-20, 21-25, 26-30, and >31, VI success was 94%, 83%, 40%, 0%, and 0%, respectively. Only 52 rrt-PCR positive samples were available for BTV, but VI results mirrored those for EHDV. At Ct-value ranges of <16, 16-20, 21-25, 26-30, and >31, VI success was 100%, 80%, 15%, 7%, and 0%, respectively. For both EHDV and BTV, VI success dropped greatly at Ct-values >21 and VI was unsuccessful with Ct-values >25 for EHDV and >27 for BTV. Results are shown in Figure 1.

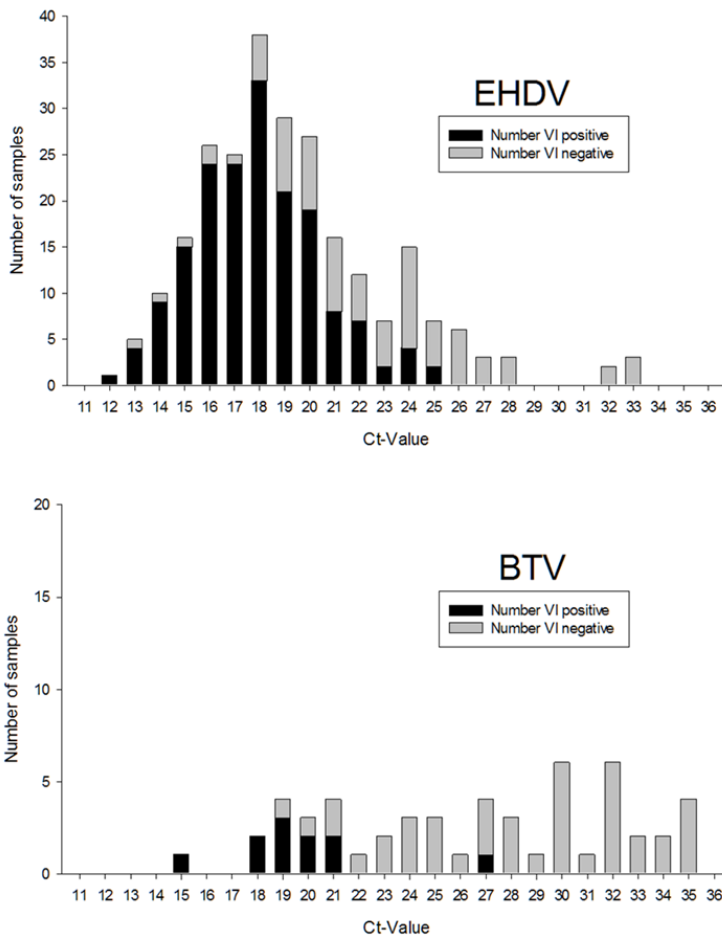


Figure 1. Comparison of Ct-values and virus isolation. Graphs show Ct-values (generated by rrt-PCR) by number of samples with corresponding number of virus isolation (VI) confirmed positives for EHDV and BTV.

Hemorrhagic disease (HD) in white-tailed deer is characterized by variable outcomes, and there are several scenarios that are worth highlighting here that make interpretation of diagnostic results such as these difficult. These variable clinical outcomes range from acute (rapid) death to inapparent (subclinical) infection. With this in mind, it is

important to note that when the virus is detected through either of the above tests, the infection may or may not represent the actual cause of death. Because of this, interpretation of diagnostic results should be supported with case histories and gross necropsy findings that are consistent with HD mortality/morbidity. Another outcome involves a very prolonged viremia (presence of virus in the blood) that can be detected for over a month after initial infection. Deer may survive infection and may or may not develop the classic post-infection hoof and rumen lesions associated with the chronic form of HD. When deer develop these secondary lesions, loss of nutritional condition and secondary bacterial infections may also result in mortality. During the course of these infections, the period of time when viral RNA can be detected by rrt-PCR exceeds the period when virus can be isolated from tissues. However, in some cases virus can be detected by both diagnostic tests for extended periods of time. The final consideration relates to herd immunity. High rates of previous EHDV and BTV infections in deer in some areas of the United States (e.g., Texas, Florida, Southern Coastal Plain) may result in >80% of the herd having existing immunity (i.e., circulating antibodies against EHDV/BTV). As a result, even if infected, these deer may experience limited morbidity/mortality. In consideration of the above mentioned scenarios, the final diagnostic assessment can be complicated; but, based on this data set we recommend the following interpretation of our combined rrt-PCR and VI results.

EHDV

PCR(+) (Ct<21)/VI positive or negative: If the deer is dead or moribund and case history is consistent with HD, it is likely that the deer is in the early stages of infection with EHDV. Infection is confirmed by VI (positive VI) or not confirmed (negative VI). Failure to confirm by VI can result from poor sample quality (decomposition), sample degradation from freeze-thaw cycles, or subtype or strain variability related to replication in cell culture.

PCR(+) (Ct 21-26)/VI positive or negative: The deer is in the later stages of infection and infection is confirmed (positive) or not confirmed (negative) by VI. If case history and clinical observations are consistent with HD, mortality can be attributed to infection.

PCR(+) (Ct 26-30)/VI negative: The deer was infected with EHDV during that year’s HD season

but likely sampled late in infection. HD is a possible cause of death, especially if lesions consistent with chronic HD (e.g., hoof lesions, rumen scars, emaciation) were observed.

PCR(-) (Ct >30)/VI negative: There is no evidence of EHDV infection.

BTV

At present, our two-year BTV data set is not robust enough to fully provide precise interpretation but based on the similar rrt-PCR and VI results, we use the following interpretation criteria for BTV. The distribution of Ct-values also differs from EHDV and includes many more samples in the higher Ct-value categories. This may be a function of lower viremia levels observed in BTV infected animals, or may relate to geographic distribution as most BTV infected animals are submitted from areas with high levels of herd immunity.

PCR(+) (Ct <21): If the deer is dead or moribund and case history is consistent with HD, it is likely that the deer is in the early stages of infection with BTV. Infection is confirmed by VI (positive VI) or not confirmed (negative VI). Failure to confirm by VI could have resulted from sample quality, or virus variability related to replication in cell culture.

PCR(+) (Ct 22-30)/VI positive or negative: The deer is in the later stages of infection and was confirmed (positive) or not confirmed (negative) by VI. If case history and clinical observations are consistent with HD, mortality can be attributed to infection. HD is a possible cause of death, especially if lesions consistent with chronic HD (e.g., hoof lesions, rumen scars, emaciation) were observed.

PCR(-) (Ct >30)/VI negative: There is no reliable evidence of BTV infection. We cannot discount or confirm a BTV infection at this range.

There is more work to be done and expansion of both the EHDV and BTV data sets during future HD seasons will allow for additional refinement of these test interpretation criteria. We will also investigate a *Culicoides* cell line (W8A) as a means to improve the sensitivity of VI especially in relation to BTV. This work is an example of the added value of our annual HD diagnostic work. With the numerous samples we receive from wildlife management agencies, we can not only provide a diagnostic workup but have the field samples we need to improve both diagnostic strategies and the interpretation of results. Thank you for your

continued support. (Prepared by Dave Stallknecht and Becky Poulson)

CWD in Captive Cervids, May 2022

In the January 2022 issue of the SCWDS BRIEFS we covered chronic wasting disease (CWD) developments in free-ranging cervids. In the current issue, we will look at CWD occurrences over the past two years in the captive cervid industry. As of May 11, 2022, CWD has been confirmed in 187 captive cervid facilities in 18 states and in 140 facilities in the Canadian provinces of Alberta, Quebec, and Saskatchewan. These numbers were provided by the USDA-Animal and Plant Health Inspection Service (APHIS) and the National Wildlife Health Center, United States Geologic Survey.

For Fiscal Year (FY) 2021, (October 1, 2020 - September 30, 2021), CWD was confirmed in 35 new captive cervid herds, eight (22.9%) of which were certified by the cooperative operator/state/APHIS CWD Herd Certification Program (HCP) as being at low risk of having CWD. All but two of the herds contained only white-tailed deer (WTD; *Odocoileus virginianus*). Thirteen affected herds were in Pennsylvania (PA), seven were in Texas (TX), six were in Wisconsin (WI), three were in Michigan (MI), two in Minnesota (MN), and single affected herds were found in Kansas (mule deer; *O. hemionus*), Montana, South Dakota (SD, elk; *Cervus canadensis*), and West Virginia (WV). Seven of the affected herds have been depopulated; the remaining 28 herds were quarantined according to APHIS.

Some of the FY2021 herd detections increased the concerns of state wildlife agencies regarding the risks that infected captive cervids potentially pose to free-ranging deer, elk, and moose (*Alces alces*). In MN, the Department of Natural Resources (DNR) issued a press release on May 25, 2021, stressing urgency in addressing CWD in the state. This was in response to a press release on the same day from the MN Board of Animal Health (BAH) announcing that 12 of 55 depopulated WTD had tested positive for CWD during its ongoing investigation of a facility in Beltrami County. This captive herd was quarantined in October 2020 because it had received deer from a Winona County source herd. A doe in the Beltrami County herd subsequently tested CWD-positive in early April 2021. While investigating the Beltrami

premises, a BAH agent discovered that the owner of the quarantined herd had moved carcasses of several deer that died during the winter to nearby county-managed, tax-forfeited land. Testing of remaining material from the decomposed carcasses by the sensitive real time - quaking induced conversion (RT-QuIC) method yielded multiple positive CWD results from numerous bones and soil samples collected at the site. The DNR subsequently built a 10-foot woven wire fence around 12 acres at the dumpsite to prevent access to CWD-contaminated material by free-ranging deer and humans at a cost of \$194,000 to the agency. Litigation is pending regarding payment by the captive herd owner to the state to cover those fencing costs as well as maintenance of the fence for 20 years. So far, follow-up testing of 2,242 wild deer samples collected in the area by the MN DNR and the tribes has yielded "Not Detected" results.

In a press release dated October 11, 2021, the MN DNR announced that it was temporarily banning captive deer movement into and within the state to protect its wild deer herd. Regulatory authority over captive cervids in MN is shared between the DNR and BAH by legislative action. The movement restriction was in place during the epidemiological investigation of two MN facilities that received five deer from a WI facility that had shipped 387 captive WTD to facilities in seven states, including two facilities in MN, in the five years prior to detection of CWD there. Two of those deer went to MN farms that no longer were in business, and the deer had been returned to WI by the time of the investigation. The three deer that remained in MN all tested "Not Detected" for CWD, and the captive cervid movement restrictions were lifted in view of the results.

Also in early October 2021, the Texas Parks and Wildlife Department (TPWD) issued a press release reminding hunters and landowners to be mindful of CWD ahead of deer season because new CWD cases had been discovered in multiple counties. The disease was detected in a free-ranging WTD in Lubbock County and in seven captive WTD breeding facilities in Duval, Hunt, Mason, Matagorda, and Uvalde counties. The TPWD was concerned that CWD could have been introduced into free-ranging deer herds on properties that received deer from the seven facilities. More than 1,700 deer were released into

high fence pastures in 119 counties across the state with more than 1,000 deer released from CWD-positive breeding facilities since 2018.

So far, in FY2022 CWD has been detected in 15 new captive cervid herds. The confirmations began on October 5, 2021, and the most recent detection was on May 11, 2022. Four (26.7%) herds were certified by the HCP as being at low risk of having CWD. Seven of the herds were in PA; six herds contained WTD and one contained red deer (*Cervus elaphus*). Three herds were in WI (two WTD herds and a mixed WTD and elk herd), two herds were in MI (one WTD herd and one elk herd) with single herds detected in Colorado (elk), SD (mule deer), and WV (WTD). The elk herd in MI has been depopulated, and the other twelve herds were placed under quarantine according to APHIS.

This update was prepared by John Fischer with assistance from Drs. Kelly Straka and Michelle Carstenson of the MN DNR, with information from

APHIS

(https://www.aphis.usda.gov/animal_health/animal_diseases/cwd/downloads/status-of-captive-herds.pdf),

MN BAH

(https://www.bah.state.mn.us/news_release/),

MN DNR

(<https://www.dnr.state.mn.us/news/index.html>),
and

TPWD

(<https://tpwd.texas.gov/newsmedia/releases/>).

SCWDS News: Student Awards and Recognition

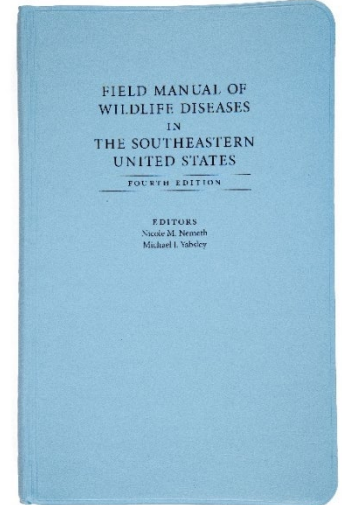
SCWDS is fortunate to have a long history of hardworking and dedicated people who have worked and/or trained here. Our current students no exception. It has been a couple of years since we highlighted the well-deserved recognitions of our graduate students. We are very proud of our students who continue to do great things and who always do a wonderful job representing SCWDS and the University of Georgia. Listed below are notable achievements:

- **Dr. Melanie Kunkel:** UGA CVM Summer Research Grants for Doctoral Students (2021) and Dissertation Completion Award (2022)

- **Dr. Chloe Goodwin:** UGA CVM Summer Research Grant for Doctoral Students (2022). and Dennis Sikes Scholarship in Experimental Pathology (2022)
- **Dr. Alisia Weyna:** UGA CVM Sidney Ewing Student Scholarship Award (2022)
- **Julia Silva Seixas:** Stoddard-Burleigh-Sutton Award, UGA (2022); Martha Love May Memorial Scholarship, Warnell SFNR (2022); Robert J. Warren graduate Fellowship, Warnell SFNR (2021)
- **Patty Torres:** Martha Love May Memorial Scholarship, Warnell SFNR (2022); The Helminthological Society of Washington Underrepresented Minority Student Grant (2022); Class of 1958 Sidney A. Ewing Graduate Scholarship, UGA CVM (2022)
- **Alec Thompson:** Class of 1958 Sidney A. Ewing Graduate Scholarship, UGA CVM (2022)
- **Morgan Friedman:** Class of 1958 Sidney A. Ewing Graduate Scholarship, UGA CVM (2022); Graduate School Communication of Research and Scholarship Grant, UGA (2022)
- **Kayla Garrett:** Byrd-Dunn Presentation Award, Southeastern Society of Parasitologists Conference (2022); Class of 1958 Sidney A. Ewing Graduate Scholarship, UGA CVM (2022)
- **Dr. Raquel Francisco:** Clarence Lee Rhodes, Jr. Graduate Fellowship, UGA (2022)

New Edition of the Field Manual is Here!

We are excited to announce the recent publication of the Fourth Edition of the *Field Manual of Wildlife Diseases in the Southeastern United States*. We thank the 17 current and former SCWDS personnel who authored sections, but especially Nicole Nemeth and Michael Yabsley who served as editors for this edition.



You will recognize familiar aspects to this new edition along with some obvious differences. Although we have moved on from the silver/grey cover to a more colorful option, the 4th Edition still has durable page and cover material suited for life in the field, not on a bookshelf. In addition to routine updates to content from the 3rd Edition, multiple additions have been made to the content. For example, new species/species group sections (e.g., bats, raptors, herpetofauna) or new diseases in existing sections (e.g., rabbit hemorrhagic disease) have been added. Despite additional content, the 4th Edition has a comparable page count because we made it slightly taller than previous editions. Throughout the book you will notice updated and improved photographs, as well as beautifully illustrated parasite life cycles by a former graduate student, Henry Adams. We have begun providing books to agencies in the Cooperative but books are also available for order on the SCWDS website under the “SCWDS Resources” tab. (Prepared by Mark Ruder)

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Information on SCWDS and recent back issues of the *SCWDS BRIEFS* can be accessed on the internet at <https://vet.uga.edu/scwds>.

Beginning January 2023, the BRIEFS will only be available online and distributed by email. In anticipation of this change, please 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.
