SCWDS BRIEFS

A Quarterly Newsletter from the

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EHDV-6 Studies at SCWDS

It has been nearly a decade since the first detection of epizootic hemorrhagic disease virus serotype 6 (EHDV-6) in the United States. During early autumn 2006, EHDV-6 was isolated from six dead white-tailed deer (two from Indiana and four from Illinois), as reported in the SCWDS BRIEFS (Vol. 23, No. 2). Subsequent genetic analysis of these viruses revealed that the EHDV-6 circulating in the U.S. was a genetic reassortant between EHDV-2 (endemic) and EHDV-6 (exotic). This means that two viruses (EHDV-2 and EHDV-6) swapped genes, most likely within a co-infected host or vector, to create a new reassortant virus, a well-known phenomenon for segmented RNA viruses, like EHDV, bluetongue virus (BTV), and avian influenza virus.

This novel EHDV-6 quickly grabbed the interest of animal health professionals for numerous reasons: 1) EHDV-6 was considered exotic to the U.S. and was not known to circulate in the Western Hemisphere prior to 2006, 2) EHDV-6 associated disease outbreaks in cattle recently had occurred in parts of the Middle East and Africa, 3) the potential impact of a novel EHDV on white-tailed deer populations was unknown, and 4) this novel virus was a genetic reassortant. Therefore, in addition to the genetic characterization of the virus, SCWDS embarked on a series of studies to better understand the outcome of infection in potential hosts (white-tailed deer and domestic cattle) and in *Culicoides sonorensis*, a confirmed vector of other EHDV serotypes.

To evaluate the pathogenicity of EHDV-6, we experimentally infected five white-tailed deer and four Holstein cattle (three cows and one steer). Animals were monitored daily for signs of disease and blood was collected for virology, serology, and clinical pathology tests. All five deer showed moderate to severe disease and three died. Viremia profiles, clinical signs and necropsy lesions in these deer were identical to those observed in deer infected with our endemic EHDV and BTV serotypes. This is consistent with observations from the field and reinforces the importance of investigating epizootic mortality in deer during vector season with diagnostic tests capable of capturing unique viruses. Only two of the four cattle had a detectable viremia, and one cow failed to seroconvert. No clinical signs were observed, which is consistent with previous experimental infections of cattle. Based on our findings, cattle are susceptible to infection with EHDV-6, but viremia was inconsistent.

Experimental EHDV infections in cattle repeatedly have failed to replicate disease reported during natural outbreaks. However, this should not lead us to dismiss EHDV as a potential pathogen of cattle, nor the potential role of cattle in EHDV epidemiology. During the severe EHD outbreak in deer in 2012, EHDV-6 was detected in cattle from several midwestern herds with animals exhibiting signs consistent with EHD. The apparent disconnect between experimental and natural conditions remains a significant hurdle in controlled infection studies.

To evaluate vector susceptibility, colonized *C. sonorensis* midges provided by the Arthropod-Borne Animal Diseases Research Unit (USDA-ARS) were allowed to feed through an artificial membrane on white-tailed deer blood spiked with EHDV-6. From 10-14 days post-feeding, only 4.5% (5/111) insects had virus titers high enough to be considered competent vectors potentially capable of transmitting virus to a susceptible host. This infection rate is very low compared with EHDV-2 (44%; 35/79), an endemic EHDV. This marked variation between

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EHDV serotypes was a somewhat unexpected finding and is interesting. Such variation has not been investigated with EHDV, but it is known that field and laboratory populations of *C. sonorensis* can vary in their susceptibility to different BTV serotypes. Additional studies are needed to understand the importance (if any) of *C. sonorensis* in EHDV-6 transmission in nature, although our findings suggest *C. sonorensis* may not be the primary vector. This is further supported by the fact that in the eastern half of the U.S., where EHDV-6 has been isolated from dead white-tailed deer repeatedly, *C. sonorensis* is rare or absent. This suggests other *Culicoides* species are involved in the transmission and maintenance of EHDV-6 (as well as EHDV-1 and -2). Numerous other *Culicoides* species are suspected EHDV and BTV vectors in the eastern U.S., and future research should aim to better understand EHDV transmission in this region.

As reported in the SCWDS BRIEFS (Vol. 22, No. 3; Vol. 23, No. 4), EHDV-6 is not the only novel orbivirus recently detected in the U.S., as multiple exotic BTV serotypes have been isolated over the past 15 years. Such detections remind us these vector-borne disease systems are dynamic and raise questions including: Will these non-endemic serotypes persist in a new ecosystem and become endemic? Will these novel serotypes potentially result in more extensive disease among hosts?

Many uncertainties remain for several of the newly detected BTV serotypes. However, the story of EHDV-6 is becoming clearer and offers insight into the emergence and subsequent establishment of an exotic orbivirus in the U.S. From 2006-2011, EHDV-6 was isolated in low numbers (1-6 cases per year) from numerous midwestern states. Even during the intense and widespread 2007 EHD outbreak, EHDV-6 represented <1% (1/281) of virus isolations made by SCWDS. However, during 2012, EHDV-6 represented 29% (55/189) of isolates at SCWDS, and the virus now is distributed widely in the eastern U.S. The underlying mechanisms for this dramatic change in transmission, disease incidence, and geographic range are unknown. Future laboratory studies and field investigations hopefully will improve our understanding of EHDV-6, a virus that likely is here to stay. More information on these studies can be found in Ruder et al., 2015. Host and potential vector susceptibility to an emerging orbivirus in the U.S.: EHDV-6. *Veterinary Pathology*, http://vet.sagepub.com/content/early/2015/10/12/0300985815610387.full.pdf+html. (Prepared by Mark Ruder)

Prescribed Fire for Reducing Tick-Associated Disease Risk

The incidence and emergence of tick-borne diseases, such as Lyme disease and ehrlichioses, has increased dramatically in the past several decades. Although this increase appears to be due to numerous factors, including enhanced diagnostics and increased reporting, one of the primary drivers is thought to be changing land use and management. This hypothesis underscores the importance of understanding land management impacts on tick populations as well as identifying how to control tick populations to reduce human disease risk.

Perhaps one of the most promising methods for controlling tick populations is prescribed burning, because it can be applied on a landscape level and is relatively time and cost efficient. Additionally, prescribed burning is a well-accepted form of ecosystem management and wildfire prevention. Several past studies have been conducted on the impacts of prescribed burns on tick populations, and the results have varied. However, these studies often did not simulate real-world land management practices and/or account for other variables known to affect tick populations such as host abundance and vegetation structure.

To investigate impacts of long-term prescribed burning on tick population dynamics and human disease risk, we performed a two-year study in southwestern Georgia at multiple locations with variable histories of more than ten years of operational burn management. Additionally, other factors known to potentially affect tick abundance (e.g., host abundance, vegetation structure, and microclimate) were evaluated at each plot. Collectively, these data provided insights into the efficacy of long-term prescribed burning for tick control and also revealed

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seasonality for numerous tick species in southwestern Georgia and northwestern Florida, an area for which there currently is little or no information on ticks or tick-borne pathogens.

Ultimately, we found that over the long-term, prescribed fires significantly reduced tick populations regardless of burn interval, host abundance, vegetation, or climate, with a 98% reduction in ticks observed in sites that were managed with any type of burning. Interestingly, we did not observe the recovery of tick populations after burns that previous studies reported. Instead, ticks simply were not present (or were present at very low numbers) in these sites for the duration of our study. Furthermore, these reductions also were observed in unburned sites immediately adjacent to burned sites, indicating that burning may be impacting tick dynamics beyond the area actually being burned. Burning also affected tick species composition, with unburned areas primarily harboring the lone star tick (*Amblyomma americanum*) and burned areas primarily harboring the Gulf coast tick (*A. maculatum*). Through follow-up studies, it was determined that the forest structure and resulting harsh microclimate achieved through long-term prescribed burning (minimal to no mid-story forest types, and semi-open pine canopy leading to hotter, drier conditions) was driving these trends. Thus, in order to achieve a healthy ecosystem and/or a reduction in ticks, the need for regular prescribed fires, not singular burns, has been underscored. Results from this part of the study recently were published in the journal *Plos One* (http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0112174).

Finally, all ticks collected during this study were tested for common, human, tick-borne pathogens including *Ehrlichia* spp., *Borrelia* spp., and *Anaplasma phagocytophilum*. Burning did not significantly affect tick-borne pathogen prevalence in the remaining ticks. However, taking into account the significant reductions in tick populations, burning does reduce the density of infected ticks in an area. On average, we calculated that an individual would encounter 0.02 infected ticks per hour in areas that were burned regularly as well as in immediately adjacent areas. However, an individual would encounter 0.70 infected ticks per hour in an unburned habitat. Thus, long-term prescribed burning can significantly reduce human tick-borne disease risk. (Prepared by Elizabeth Gleim, Oxford College, and Michael Yabsley)

Heartland Virus Update

Results of serologic surveys of coyotes, moose, raccoons, and white-tailed deer in midwestern and eastern states revealed Heartland virus (HRTV) exposure over a much broader range than previously recognized. These findings, published by U.S. Centers for Disease Control and Prevention researchers in the October issue of *Emerging Infectious Diseases* (http://dx.doi.org/10.3201/eid2110.150380), increase the known distribution of HRTV from three to fourteen states, support the suspected role of the lone star tick (*Amblyomma americanum*) as the primary vector, and provide a better understanding of the risk of human infection. Antibodies to HRTV were demonstrated for the first time in coyotes and moose and were reconfirmed in raccoons and white-tailed deer, suggesting that future surveillance should involve a wide variety of wildlife species.

Heartland virus, a newly described member of the Bunyaviridae family, is a tick-borne pathogen that first caught the attention of scientists, physicians, and public health officials when it was isolated from the blood of two sick men in northwestern Missouri in 2009 (SCWDS BRIEFS Vol. 28, No. 3). To date, there have been nine reported human cases of HRTV-associated disease in Missouri, Oklahoma, and Tennessee, including two deaths. However, HRTV has not been associated with disease in, or isolated from, wild or domestic animals.

The known range of the lone star tick includes portions of the Midwest, Southeast, and Northeast, and HRTV serosurveillance targeting wild mammals was conducted in this region between 2009 and 2014. Banked blood from healthy, live-trapped, hunter-harvested, culled, or road-killed coyotes, moose, raccoons, and white-tailed deer from 19 states within the known *A. americanum* range were analyzed for HRTV-neutralizing antibodies; one or more of these four wildlife species tested seropositive in

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13 of the 19 states. These results expand the known range of HRTV from Missouri, Oklahoma, and Tennessee to include Florida, Georgia, Illinois, Indiana, Kansas, Kentucky, Maine, New Hampshire, North Carolina, Texas, and Vermont.

The area covered by the states with seropositive wildlife largely parallels the range of the lone star tick; however, seropositive white-tailed deer also were found in Maine, New Hampshire, and Vermont, where *A. americanum* is not known to be established. Possible explanations include unreported lone star tick populations in these areas, alternative vector(s), immigration of seropositive animals into these states, or the presence of antibodies to a closely-related virus that cross-react serologically with HRTV.

Heartland virus has been isolated only from lone star ticks in northwestern Missouri. The detection of HRTV in unfed nymphs suggests they may become infected as larvae feeding on viremic, vertebrate hosts of the species in which antibodies have been found as well as in other species. Serological surveys of white-tailed deer and raccoons in northwestern Missouri found high HRTV antibody prevalence in both species, indicating they are suitable targets for HRTV serosurveillance.

The significance of HRTV as an emerging pathogen is not yet known, and its potential impacts on wildlife are unclear. Although this new information expands the apparent range of HRTV, it does not clarify the geographical dispersal of HRTV over time, or precisely when and how HRTV might have been circulating historically in these areas. From a public health perspective, HRTV remains a concern, and the new findings suggest a geographically expanded risk of exposure for humans. Further surveillance and controlled studies are needed to fill the gaps in our understanding of HRTV. (Prepared by John Bryan)

Congenital Anomalies in a Fawn

A private citizen observed a white-tailed deer fawn with unusual growths on its head in northern Virginia in June 2015. The Virginia Department of Game and Inland Fisheries was notified, and the fawn was euthanized and submitted to the Southeastern Cooperative Wildlife Disease Study for necropsy and diagnostic testing. Gross necropsy findings included a fluid-filled subcutaneous mass above the left eye (Figures 1A and B) as well as an out-pouching of tissue protruding through a 2.5 cm defect on the dorsal surface of the skull (arrows in Figures 1A and B). The right eye was shrunken and approximately 1/5 the size of the left eye (Figure 2).

Histological evaluation determined that the large mass above the left eye was a subcutaneous cyst. Microscopic examination of the herniated tissue suggested the presence of neural tissue, choroid plexus, and meningeal tissue. The structure was diagnosed as a meningoencephalocele. The right globe was diffusely shrunken and diagnosed as micropthalmia. The uveal tract of the right eye was poorly defined, with lack of proper ocular development, and no lens (aphakia). The optic nerve and outermost layers of the retina appeared to be normally developed.

Congenital ocular anomalies of white-tailed deer have been well documented, and a number of them have been observed at SCWDS. Reported anomalies include micropthalmia, anopthalmia, aphakia, retinal dysplasia, ectopic lacrimal gland, corneal dermoid, and immature iridocorneal angle structures. To our knowledge, this is the first report of ocular anomalies associated with a meningoencephalocele in a white-tailed deer. Presumably, all defects arose due to abnormal embryonic development.

Skull, neural tube, and vertebral column defects result from abnormal closure of the spine. Similar anomalies have been reported in many domestic and zoo animals as well as in humans with spina bifida. The cause of the abnormal development is unknown. There are some anecdotal reports of clusters of cases of ocular anomalies in white-tailed deer in localized areas, but overall, the condition is poorly understood. Micropthalmia in humans has been associated with an abnormal number of chromosomes, and mutations in certain genes have been associated with ocular anomalies in domestic dogs. A specific gene has not been associated

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Figure 1A.

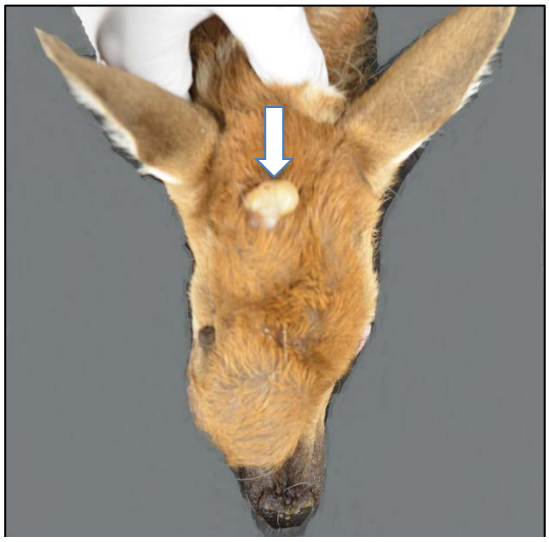

Figure 1B.

Figure 2.

with congenital anomalies in white-tailed deer. Ocular anomalies have been well described in domestic animals, including cyclopic lambs born to ewes grazing on California corn lily (*Verratrum californicum*) at 14 days of gestation. Another possible cause in the fawn is viral infection of the doe during gestation. Results of immunohistochemical staining for infection with bovine viral diarrhea virus in the fawn were negative, but exposure of the doe to other viruses cannot be ruled out. (Prepared by Heather Fenton, Megan Kirchgessner of the Virginia Department of Game and Inland Fisheries, and Kaitlin Fiske of the University of Florida)

Deer Trafficker Fined $1,600,000

The largest fine ever assessed for a wildlife crime in the United States was levied in September when a Georgia man who pleaded guilty to three Lacey Act violations was ordered to pay $1.6 million in fines and restitution. He also was sentenced to three years of probation, four months of home confinement, and 150 hours of community service. In addition, he agreed to publish a statement in *North American Whitetail* magazine.

Under the Lacey Act, it is unlawful to import, export, transport, sell, or purchase wildlife, fish, or plants that were taken, possessed, transported or sold in violation of a state, federal, or foreign law. When it was passed in 1900, the Lacey Act became the first Federal law protecting wildlife.

According to court documents, the Georgia man and a co-conspirator illegally shipped captive white-tailed deer to Florida from Ohio and attempted to ship deer to Georgia from Ohio. The attempted shipment to Georgia was intercepted on an interstate highway in Ohio when Ohio Division of Wildlife officers noticed deer inside a cargo trailer. Georgia law prohibits importation of live deer. The Ohio herds where the deer originated had not been tested for chronic wasting disease, bovine tuberculosis, or brucellosis as required by Federal law for interstate shipment. In addition, the men had placed federal identification tags from a certified,

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disease-free deer that had died into the ear of an uncertified deer they were selling. They then sold breeding services and semen from the deer to breeders around the United States. The defendants also sold deer hunts at an improperly licensed, high-fenced enclosure they co-owned in Ohio, charging $1,000 - $50,000 to kill deer inside the fence.

The co-conspirator, who pleaded guilty to 12 charges related to violating the Lacey Act, one count of conspiracy and one count of wire fraud, was sentenced to 21 months in prison, a $125,000 fine, and 200 hours of community service. He also was ordered to publish an article in *The Deer Breeders Gazette*. A third defendant, who earlier pleaded guilty to eight charges related to offering illegal hunts in violation of the Lacey Act, was sentenced to four months of house arrest and three months of probation. (Excerpted from a September 15, 2015, press release from the U.S. Attorney's Office, Southern District of Ohio at http://www.justice.gov/usao-sdoh/pr)

SCWDS News

SCWDS continues to have a strong training program and has graduated a number of new scientists since our last update. Dr. Jennifer Ballard defended her Ph.D. on the epidemiology of Wellfleet Bay virus in common eiders and has started a position in the Wildlife Health Office of the United States Fish and Wildlife Service's National Wildlife Refuge System Program in Fort Collins, Colorado. Christopher Cleveland completed his M.S. in May 2015, focusing on the impact of environmental factors on *Culicoides* spp. communities in the southeastern U.S. and potential relationships with the geographical distribution of hemorrhagic disease. Chris is a Research Professional at SCWDS and has begun work on a Ph.D. project. Sarah Coker completed her M.S. in 2015 on hemoparasites of white ibis and now is working as a SCWDS Research Technician. Elizabeth Gleim received her Ph.D. in May 2013 after completing her research on prescribed fire effects on tick populations. Since graduation, Liz has been working as a visiting Assistant Professor of Biology at Oxford College of Emory University in Oxford, Georgia, and she continues to conduct research on the ecology of ticks and tick-borne pathogens. Other students who graduated in 2014 and 2015 include Whitney Kistler (Ph.D.), Kimberly McDermid (M.S.), Albert Mercurio (M.S.), Andrew Ramey (Ph.D.), and Barbara Shock (Ph.D.). SCWDS is very proud of our graduate students, and we wish them well in their future endeavors.

There are new faces at SCWDS, including one who may be familiar to you. Dr. Mark Ruder recently returned to SCWDS as an Assistant Research Scientist. Mark received his Ph.D. in 2012 after completing his work on hemorrhagic disease viruses in deer and cattle at SCWDS. He then became a Research Veterinary Medical Officer for the Arthropod-Borne Animal Diseases Research Unit (USDA-Agricultural Research Service), where he focused on *Culicoides* ecology and the epidemiology and pathobiology of numerous arthropod-borne viruses of livestock and wildlife. Mark plans to continue similar research at SCWDS and has re-joined the diagnostic service. We welcome him back.

Dr. Charlie Bahnson recently started as a diagnostician and Ph.D. student. He is a South Dakota native and worked for Montana Fish, Wildlife, and Parks prior to attending veterinary school at Iowa State University. He looks forward to gaining experience at SCWDS and hopes to eventually work for a state wildlife management agency.

Faculty, students, and staff at SCWDS have been recognized locally and internationally for the great work that they do. Dr. Sonia Hernandez recently received a highly competitive National Science Foundation Ecology of Emerging Infectious Diseases grant for $2.1 million to investigate anthropogenic impacts on birds over the next five years. The objectives of this project are to examine how use of anthropogenic resources, such as supplemental feed, influences pathogen dynamics across organizational scales, from the colonization of individual hosts to transmission across the landscape. This research explores interactions between an enteric pathogen, *Salmonella*, and the American white ibis (*Eudocimus albus*) in southern Florida, in order to understand how resource shifts in urban

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habitats alter host ecology and pathogen dynamics. Dr. John Fischer received the 2015 Charles Dobbins Award for Excellence in Service from the UGA College of Veterinary Medicine for his leadership of SCWDS and his service to the wildlife community.

Post-doctoral fellow Dr. Neus Latorre-Margalef received a Cross-Network Training Program grant through the National Institute of Health's Centers for Excellence in Influenza Research and Surveillance (CEIRS) to conduct collaborative research on avian influenza virus at the Erasmus Medical Center in the Netherlands. Dr. Betsy Elsmo, a SCWDS diagnostician and pathology resident, received a $1,500 travel award from the American Association of Veterinary Laboratory Diagnosticians to attend its 2015 meeting in Providence, Rhode Island. She will present "Acute, fibrinohemorrhagic, interstitial pneumonia and suppurative myocarditis associated with *Bartonella henselae* in three Florida panthers (*Puma concolor coryi*)."

Todd Nims recently was selected as one of the few faculty and students invited to attend the fourth International Workshop on Malaria and Related Haemosporidian Parasites of Wildlife in Shepherdstown, West Virginia. Todd is a Ph.D. student working with Dr. Michael Yabsley on the ecology of blood parasites in aquatic turtles. He completed his M.S. at Georgia Southern University on the effects of prescribed fire on parasites of rodents.

Sarah Sapp won the "Best Student Poster Award" at this year's Wildlife Disease Association (WDA) meeting in Australia. Sarah is a Ph.D. student who received her bachelor's degree in biology from Berry College. Her doctoral work focuses on raccoon roundworm (*Baylisascaris procyonis*). She also received third place for her oral presentation at the annual symposium of the UGA student chapter of WDA. Sarah Coker, SCWDS Research Technician and former M.S. student, received third place for her poster titled "Prevalence and diversity of *Haemoproteus* spp. in the American white ibis in southern Florida" at the same symposium.

Joshua Thomas was chosen as one of the "Top 100 UGA Student Employees of the Year" out of thousands of student workers. Josh currently is studying forestry economics and assisting SCWDS with administrative and financial duties. Congratulations to all of our faculty, students, and staff, all of whom are very deserving of the awards and recognition they have received. (Prepared by Heather Fenton)

USAHA Resolutions Involving Cervids

Three resolutions potentially impacting captive and/or free-ranging cervids were adopted by the United States Animal Health Association at its 2015 meeting in Rhode Island:

- The United States Animal Health Association (USAHA) urges USDA-Veterinary Services (VS) to amend the Chronic Wasting Disease (CWD) Program Standards by deleting all language in Part B (Guidance on Responding to CWD Affected Herds) and rewriting Part B under the guidance of a working group of state and federal regulatory officials and representatives from the farmed cervidae industry.

- The USAHA urges USDA-VS to work with stakeholders to develop a guidance document on determining CWD risk levels of source herds for interstate cervid restoration projects.

- The USAHA urges USDA-VS, as well as State Animal Health Officials, to continue and expedite discussions and evaluation of ante-mortem collection procedures for medial retropharyngeal lymph node (MRPLN) tissues for live animal testing for CWD in white-tailed deer. Veterinary Services is also urged to issue a VS guidance document stating that ante-mortem collection procedures for MRPLN tissues are acceptable and authorized in accordance with current laws (9 CFR 55 and 9 CFR 81) and existing Federal CWD Program Standards (MAY 2014).

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