

SCWDS BRIEFS

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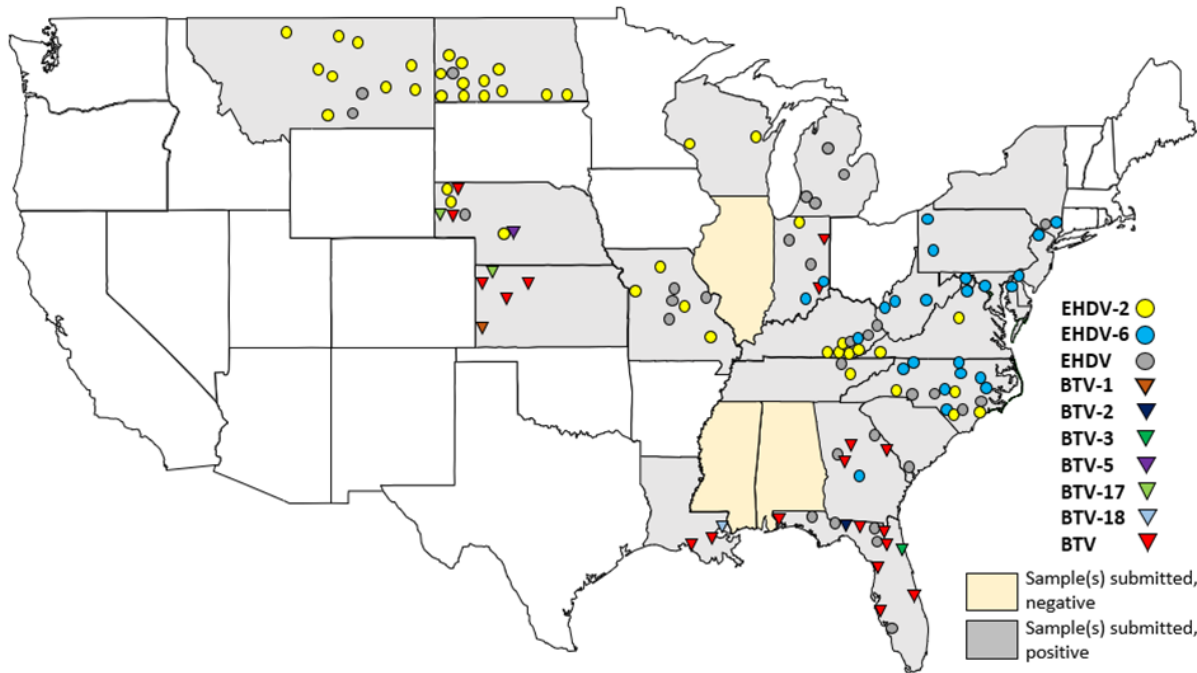


Figure 1. Map showing the distribution of EHDV and BTV detections by SCWDS during 2020. Some symbols may represent more than a single detection.

2020 Hemorrhagic Disease Summary

As was highlighted in the [October 2020 issue of the SCWDS BRIEFS](#), SCWDS has provided annual diagnostic testing for epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) for the last 30 years. Annually, we receive 200-400 submissions from state wildlife management agencies, as well as some agriculture agencies and veterinary diagnostic laboratories. Most submissions consist of tissue samples (typically lung and/or spleen) from wild white-tailed deer, although a minority come from other wild and captive ruminants. Samples are screened for EHDV and BTV using real-time reverse-transcription polymerase chain reaction (rRT-PCR) assays, and virus isolation is attempted on positive samples. Virus isolates are further identified to serotype. While the identification and isolation of these

viruses does not provide the complete picture of EHDV and BTV activity in wild ruminants in the US, the data do provide situational awareness on ongoing outbreaks for wildlife agencies, a means to validate parallel annual data provided by our Annual National Hemorrhagic Disease (HD) Survey, and the isolates represent valuable resources for future research.

Emerging viruses (i.e., SARS-CoV-2, rabbit hemorrhagic disease virus 2) were unfortunately a common theme in 2020. Although HD is certainly not a new disease in North America, we have clearly observed changing dynamics over the last two decades, such as the detection of new EHDV/BTV serotypes and changing epidemiologic patterns (e.g., increasing expansion in the northern US). In this sense, HD is an emerging disease in some regions of North America. Amid restrictions

Continued...

associated with the COVID-19 pandemic, many agency staff were limited in their ability to investigate deer mortalities. Despite these limitations, biologists were still able to submit a large number of samples to SCWDS, and diagnostic results from 2020 clearly show the continuation of some of the changing patterns mentioned above.

During 2020, SCWDS received 274 submissions for EHDV and BTV diagnostic testing from 25 different states. The vast majority of submissions (239/274) were from white-tailed deer, although we also received tissue samples from 16 pronghorn, 13 mule deer, three elk, two bighorn sheep, and two moose. Overall, we had 162 virus detections (128 EHDV and 34 BTV) from 22 states, including 86 virus isolates representing numerous serotypes (EHDV-2, -6, and BTV-1, -2, -3, -5, -17, and -18) (see Figure). As is the case every year, most detections were from white-tailed deer and EHDV-2 was the most common virus identified (42 isolations from 10 states). However, other ruminants were involved in some regions. In particular, EHDV-2 was isolated from white-tailed deer, mule deer, pronghorn, and elk in Montana and/or North Dakota. Additionally, BTV-17 was isolated from bighorn sheep (Nebraska) and mule deer (Kansas), and BTV-1 was isolated from a pronghorn (Kansas). Aside from the diversity of hosts represented, the diversity of viruses recovered from Kansas and Nebraska was notable. In these two states we detected two common and historically endemic viruses (EHDV-2 and BTV-17), as well as two historically exotic viruses (BTV-1 and BTV-5) that had not been previously documented in this part of the country. Further, a white-tailed deer in Nebraska was co-infected with EHDV-2 and BTV-5. The outbreaks in the Great Plains during 2020 highlight the complexity of hemorrhagic disease in North America – a system that involves multiple viruses, multiple ruminant hosts, and likely multiple *Culicoides* vector species.

Moving into the eastern United States, all EHDV and BTV detections were in white-tailed deer. Much of the HD activity in the eastern US was driven by EHDV-6 (27 virus isolates from 11 states), especially in the mid-Atlantic and northeastern states. This included the first reports of EHDV-6 in New York, Delaware, and Georgia. Another trend that is apparent when examining the 2020 map is the continued expansion of HD into the upper Midwest and Northeast (e.g., Wisconsin, Michigan,

and New York). A further example of this phenomenon is West Virginia, a state with a 5-6 year HD outbreak cycle historically but that has now had annual outbreaks for 5 consecutive years (2016-2020). Work is needed to better understand the potential underlying mechanisms that may explain these observed changes. Finally, moving deeper into the Southeast, we isolated additional BTV serotypes, including BTV-2 and BTV-3 in Florida and BTV-18 in Louisiana. Both BTV-3 and BTV-18 represent historically exotic BTV serotypes. This high diversity of viruses observed in the southernmost states like Louisiana and Florida is expected and is likely associated with their unique habitats and climate, which in turn drive vector population dynamics. For example, in addition to the historically endemic EHDV and BTV serotypes known to occur in the United States, over 10 exotic viruses have been confirmed in Florida over the last 20 years. This region of high virus diversity may serve as a potential source of virus for other parts of the country via movement of infected hosts or vectors across the landscape.

We thank the many wildlife professionals who submitted tissue samples for diagnostic testing this past season and are grateful that you continued to contribute samples in spite of interferences related to the COVID-19 pandemic. The data obtained through such effort are critical to documenting the viruses associated with HD outbreaks in wild ruminant populations throughout much of the US and help to better document and understand the changing patterns of HD. (Prepared by Mark Ruder, Natalie Stilwell and Dave Stallknecht)

COVID-19 and Wildlife

It has now been a year since the first human cases of a novel coronavirus infection known as SARS-coronavirus-2 (SARS-CoV-2) surfaced in Asia and spread rapidly in human populations around the globe. The pandemic has resulted in more than 105 million human cases worldwide and infection rates are still rising in many areas. During this pandemic, several questions have emerged regarding the potential role of wildlife. These questions have primarily related to the origin of this virus and the susceptibility of wildlife species to SARS-CoV-2.

Early genetic analyses suggest SARS-CoV-2 likely originated after spilling over into human populations from an animal reservoir, but the exact source and species remain unknown. The virus appears

genetically and structurally similar to bat and pangolin coronaviruses, suggesting that one or both species played a role in evolution of the virus prior to transmission into humans. While wildlife may be the initial source of SARS-CoV-2 exposure to humans, zoonotic transmission was likely facilitated by actions related to the commercialization and utilization of wildlife. Similar transmission patterns have occurred in the past with the highly pathogenic SARS- and MERS-coronavirus outbreaks, which arose from human contact with horseshoe bats and camels, respectively. To further elucidate information on the source of SARS-CoV-2, the World Health Organization recently deployed a team of scientists to Wuhan, China, where the first human infections were identified in connection with a live animal market.

Meanwhile, researchers around the world are focusing on SARS-CoV-2 susceptibility in different hosts through two main methods: 1) examining the angiotensin-converting enzyme 2 (ACE2) receptor, and 2) performing live animal experimental infection studies. The ACE2 receptor, which is the host cell binding site for the coronavirus spike protein, varies significantly among animal species and its structure implies whether SARS-CoV-2 can attach and enter host cells. Experimental infections go one step further to evaluate *in vivo* susceptibility, along with pathology, virus transmission and shedding patterns, which can provide insight into how SARS-CoV-2 behaves in individual animals.

So far, published studies show that certain bat, rodent, felid, rabbit, mustelid, non-human primate, skunk, bovid, canid, and deer species demonstrate a degree of susceptibility to SARS-CoV-2 infection, whereas other rodents and all examined bird, pig, insect vector, raccoon, and North American bat species were not susceptible. Regarding wildlife, a recent study conducted by the USDA Agricultural Research Service examined the potential for experimental infection in white-tailed deer. In this study, fawns were found to be susceptible to intranasal inoculation with a high dose of SARS-CoV-2. Although infected fawns remained asymptomatic, viral transmission to naïve, cohabitating fawns was documented. A follow-up study will further examine transmission and shedding patterns in white-tailed deer.

While susceptibility studies give some idea of the relative risk of infection in various species, these data only provide partial evidence that a species

could act as a potential animal reservoir for SARS-CoV-2. Results from controlled laboratory studies are also insufficient to predict population-level disease risks in the natural environment. Therefore, to put these susceptibility studies into perspective it's important also to focus on natural infection data. To date, SARS-CoV-2 has been detected in a limited range of non-domestic species, including felids (i.e., lion, tiger, snow leopard, puma), mustelids (i.e., mink, ferret), and non-human primates (i.e., gorilla). The most extensive effects have been observed in farmed mink. According to recent CDC figures, SARS-CoV-2 infection has been confirmed at more than 400 mink farms worldwide; most cases have occurred in Denmark and the Netherlands where the fur industry is prevalent and individual facilities house thousands of animals. Many facilities were depopulated due to the observed high infection rate and subsequent risk of zoonotic spillover to farm personnel, along with the detection of novel, mutated variants of the virus in some cases. Similar outbreaks have occurred on mink farms in the United States. Although these cases were dramatic and widely publicized, it's important to remember that outbreaks in high-density, industrial facilities such as those used for mink farming do not directly correlate with potential risks to wild populations, as captive animals are typically held in artificial conditions where they have high contact rates with humans and other animals. Farmed animals may also undergo generations of selective breeding for desired characteristics, such as fur color and quality in mink, which can result in decreased genetic diversity and impaired immune function compared to their wild counterparts.

Aside from farmed mink, natural infections in other animal hosts have rarely been reported, which suggests the vast majority of animal species are poorly susceptible to SARS-CoV-2. For example, a very low number of SARS-CoV-2 cases have been reported in domestic dogs (n=41) and cats (n=53) in the United States. These numbers are particularly striking considering there are more than 150 million dogs and cats in US households. In non-domestic animals, infections (while still rare) have largely been restricted to animals in captivity. Notable exceptions in the United States have been free-ranging, wild mink or escaped farmed mink captured on or near affected mink farms in Utah and Oregon, respectively. In general, the risk of viral spread is inherently higher when animals are housed in high densities (e.g., farm facilities) or

have increased contact with humans (e.g., in zoological or rehabilitation settings), compared to in natural environments. In contrast, there is no evidence yet to suggest SARS-CoV-2 infection can be sustained in wild animal populations.

Infection rates over the past year show that COVID-19 is largely a disease affecting humans, not animals. Efficient human-to-human transmission has been the single most important driver of the global spread of SARS-CoV-2. Furthermore, SARS-CoV-2 introduction into animal populations has occurred only under specific conditions involving direct contact with infected humans. Still, appropriate measures need to be taken to minimize viral transmission between humans and animals, not only for the consideration of the current SARS-CoV-2 outbreak but to prevent similar pandemics from occurring in the future. Regarding SARS-CoV-2, those working closely with captive or free-ranging wildlife should follow the same measures used to prevent human-to-human virus transmission, which include wearing appropriate PPE, disinfecting spaces frequently, and undergoing testing and self-isolation if COVID-19 exposure is suspected. With several SARS-CoV-2 vaccines now being deployed, widespread vaccination of humans will also play a crucial role in obtaining herd immunity and reducing viral spread in human and animal populations. (Prepared by Natalie Stilwell, Mark Ruder, and David Stallknecht)

Rabbit Hemorrhagic Disease Virus 2 Continues Lurking

In the [April 2020 issue of the SCWDS BRIEFS](#), we discussed the ongoing outbreak of rabbit hemorrhagic disease (RHD) virus 2 (RHDV2) in the southwestern United States and Mexico. At the time, RHDV2 had been reported in wild rabbits and hares in New Mexico, Arizona, Colorado, and Texas, and in domestic rabbits (*Oryctolagus cuniculus*) in New Mexico, Arizona, and Texas. As feared, the RHD outbreak continued to expand and by mid-December 2020, RHDV2 had been confirmed in wild and domestic or feral rabbits throughout a vast and largely contiguous region of the western US (Arizona, California, Colorado, New Mexico, Texas, Utah, and Wyoming). Unfortunately, in late December 2020, RHDV2 was confirmed in domestic rabbits in Lake County, Florida.

The RHD outbreak in Florida occurred in a small, non-commercial, backyard population of rabbits

bred and raised for meat. All rabbits died or were culled over a two-week period. The outbreak represents the first confirmed detection of RHDV2 in rabbits (domestic or wild) in Florida. Clinical signs included bleeding from the nose and sudden death. The location has since been disinfected and placed under quarantine and a fallow (rabbit free) period for 90 days. The backyard rabbit operation was closed, meaning no rabbits were recently imported or exported and the route of RHDV2 introduction is not currently known. However, the Florida Department of Agriculture & Consumer Services (FDACS) and United States Department of Agriculture (USDA) are conducting an ongoing epidemiologic investigation into the outbreak. The Florida Fish and Wildlife Conservation Commission and FDACS are continuing outreach efforts to educate citizens of the risk and encourage prompt reporting of sick or dead wild and domestic rabbits. To date, no additional cases of RHDV2 have been reported in Florida.

This recent RHD outbreak in Florida represents the most southeastern detection of RHDV2 in the US, with the next closest report being in domestic rabbits in central Texas. However, isolated RHDV2 outbreaks in domestic rabbits in the eastern US occurred previously in Ohio (fall 2018) and New York (spring 2020) and genetic analysis of these viruses by USDA suggests these viruses were distinct from those currently circulating in the western US and likely represent different introductions. The USDA is performing a similar genetic analysis of the RHDV2 detection from Lake County, Florida. This information will help determine the genetic relatedness of the Florida RHDV2 to other viruses detected in North America from 2018-2020, which will help understand if the Florida outbreak represents significant spread of the ongoing outbreak in the Southwest, or yet another separate introduction.

RHDV2 is a highly infectious and lethal virus that may persist in the environment for extended periods. Clinical signs may include fever, lethargy, or ocular and nasal bleeding. However, sudden death is often the only sign of infection. Virus is shed in most bodily secretions and transmission is through direct or indirect contact and the virus can remain stable in carcasses/tissues and the environment for months. With such efficient transmission and the multitude of susceptible domestic and wild lagomorph hosts, the risk of RHDV2 spread is high. Biosecurity (e.g., sanitation,

limiting movement) remains key to preventing the spread of RHDV2 among domestic and wild populations. Movement of live or dead rabbits, rabbit parts, equipment, bedding, or any other materials rabbits have contacted are significant risk factors for the spread of RHDV2 and preventive measures should target minimizing the risk of long-distance movement of the virus.

There is potential for RHDV2 to significantly impact wild rabbit and hare populations, as well as pet rabbits and production rabbits. The effects of potential wild lagomorph population declines on predator populations remains unknown but also raises concern. To date, RHDV2 has been confirmed in the black-tailed jackrabbit (*Lepus californicus*), antelope jackrabbit (*Lepus alleni*), desert cottontail rabbit (*Sylvilagus audubonii*), mountain cottontail rabbit (*Sylvilagus nuttallii*), and eastern cottontail rabbit (*Sylvilagus floridanus*), although the wild lagomorph host range in North America remains unclear and all lagomorphs should be considered susceptible until further information is gathered. Prompt detection of the virus in new areas, followed by robust disinfection and containment measures are the best chance to control outbreaks. However, once RHDV2 is circulating in wild populations, management options become limited. Increased vigilance with prompt reporting and investigation of wild or domestic rabbit mortality events are critically important, especially in unaffected areas. Robust communication and cooperation among state and federal wildlife and agricultural agencies, as well as citizen stakeholder groups, is paramount to the successful prevention and management of RHDV2 in the US.

Currently, RHDV2 is classified by USDA-APHIS-Veterinary Services as a foreign animal disease and is reportable to the World Organization for Animal Health (OIE). Therefore, close cooperation between state and federal wildlife and agricultural agencies is important. Please contact SCWDS if assistance is needed with outreach, prevention, or response activities. To submit a wild lagomorph carcass to SCWDS for RHDV2 testing, please work with our Diagnostic Service to coordinate shipment. (Prepared by Katie Vivirito, University of Illinois, and Mark Ruder)

Influenza Persistence in Waterfowl Habitats

The epidemiology of any infectious wildlife disease is dependent on interactions between host,

pathogen, and the environment. Influenza is no exception. We have known for more than 50 years that waterfowl represent an important reservoir for a genetically diverse population of influenza A viruses (IAV). However, our understanding of the interactions between host, IAV, and the environment that provide a means for IAV transmission and maintenance in wild birds is incomplete. It is widely accepted that IAV transmission in waterfowl populations primarily occurs via an indirect fecal/oral route that involves contaminated water. Infectious IAV have been isolated directly from water samples collected from waterfowl habitats, and it has been demonstrated experimentally that these viruses can remain infective in water for extremely long durations depending on water temperature and other physical and chemical properties such as pH and salinity. In a distilled water laboratory model, for example, IAV can remain infective for more than one year in water at 4°C. Most of what we know about the stability of IAV in water is derived from controlled experimental studies; validating these results in the field has proven challenging. This challenge relates to the physical, chemical, and biological complexity of natural water bodies, direct testing limitations related to the large volumes of water associated with waterfowl habitats, and biosafety concerns associated with potential field release of laboratory propagated IAV.

In a collaboration with the United States Geological Survey (USGS) Alaska Science Center, we recently developed a system to safely evaluate the infectivity of IAV in water under monitored field conditions. This system relies on the periodic testing of cloacal/oropharyngeal (CL/OP) swab material from ducks that are diluted in filter-sterilized water collected from the corresponding waterfowl habitats. The swab-inoculated water samples are contained in individual tubes within a larger barrel-type container and submerged in a natural water body for subsequent retrieval and testing. This system was originally described by Reeves et al. (2020). By retrieving and testing contained swab/water samples over time, this model system was shown to provide a safe method to assess the long-term viability of IAVs that are naturally shed by infected waterfowl into water under the physical and chemical conditions that are present in the specific habitats that ducks are utilizing.

In a more recent study, we applied this technique to waterfowl habitats in Alaska, Louisiana, and

Minnesota. The goal was to better understand the potential for IAV to overwinter in the environment in these habitats. This study was done not only with our colleagues at the Alaska Science Center but also involved collaborators from the California Water Science Center (USGS), Western Ecological Research Center (USGS), Memorial University of Newfoundland, US National Poultry Research Center (USDA), Louisiana Department of Wildlife and Fisheries, and Patuxent Wildlife Research Center (USGS). In this study, two field sites were selected from wetlands at Izembek National Wildlife Refuge (Alaska), Agassiz National Wildlife Refuge (Minnesota), and Cameron Parish (Louisiana).



Figure 2. SCWDS biologists, Deb Carter and Alinde Fojtik, deployed a submersible chamber containing CL/OP swabs at Agassiz National Wildlife Refuge (Minnesota) where they remained overwinter.

Surface water from all sites was collected, filter sterilized, chemically characterized, and divided among tubes that were filled with 40 mL of the filtered water. Individual tubes were then inoculated with a combined CL/OP swab collected from a single wild duck utilizing these wetlands and each inoculated water sample was evenly divided (13 mL each) into two replicate samples. Replicate #1 was submitted to the SCWDS laboratory for immediate IAV testing. Replicate #2 samples were sealed and placed in a larger barrel-like container and submerged for 6-7 months in the waterfowl habitat for subsequent retrieval (see photo). Sampling in Alaska and Minnesota was done in September (corresponding to early fall migration); ducks were sampled in Louisiana during November as birds arrived on wintering areas.

Of the 686 surface water samples that were inoculated with a CL/OP swab, IAV was initially

isolated from 51 (7.4%) of the replicate #1 samples. These included 40 samples from Minnesota and 11 from Alaska. IAV was not isolated from samples collected from Louisiana. Replicate #2 samples from Alaska and Minnesota were retrieved during April (approximately 6 – 7 months later). Of the 51 swab/water samples that originally yielded an IAV isolate, 10 (20%) remained infective when inoculated into eggs. These included an assortment of IAV subtypes including H2N9, H3N6, H3N8, H4N6, H4N8, and low pathogenicity (LP) H7N3. These samples had remained infective for 209 -229 days in filtered lake water under natural water temperature conditions. To confirm that the positive replicate #2 water samples still contained virus capable of infecting waterfowl, we experimentally challenged 10 groups of three mallards with approximately 0.5 mL of these water samples. Infection of mallards, as measured by three criteria: detection of viral RNA, virus isolation, and seroconversion, was observed with two of ten samples corresponding to an H3N8 and LP H7N3 IAV. The field study was further supported by a laboratory trial using the residual water in the 40 positive replicate #1 samples from Minnesota. Following initial testing, these were maintained at 4°C and tested monthly from September to April. Of these 40 samples, five (13%) remained infective for at least seven months (April represented the last month of testing for this experiment).

Results from this work confirm that IAV can persist in the natural environment during the time when waterfowl are not present, or are in greatly reduced numbers, on northern waterfowl habitats. This potentially provides a means for infectious IAV to be maintained from one IAV season to the next. Although the model system employed in these studies provides an improved and more relevant method to assess environmental stability of IAV in waterfowl habitats, it does not capture all of the biological, chemical, physical, and hydrologic factors and interactions that can exist in a natural water body. A great deal of work remains to be done to better understand and confirm the role and importance of environmental transmission in the maintenance of these viruses. With current detections and wild bird mortality associated with highly pathogenic H5 viruses of the A/Goose/Guangdong/1/1996 lineage in waterfowl in Asia, Europe, and Africa, this understanding may prove valuable if we are faced with a similar problem in North America.

For additional details related to this work see:

Ramey AM, Reeves AB, Drexler JZ, Ackerman JT, De La Cruz S, Lang AS, Leyson C, Link P, Prosser DJ, Robertson GJ, Wight J, Youk S, Spackman E, Pantin-Jackwood M, Poulson RL, Stallknecht DE. [Influenza A viruses remain infectious for more than seven months in northern wetlands of North America.](#) *Proc Biol Sci.* 2020 Sep 9;287(1934):20201680. doi: 10.1098/rspb.2020.1680.

Reeves AB, Ramey AM, Koch JC, Poulson RL, Stallknecht DE. [Field-based method for assessing duration of infectivity for influenza A viruses in the environment.](#) *J Virol Methods.* 2020 Mar;277:113818. doi: 10.1016/j.jviromet.2020.113818

(Prepared by Dave Stallknecht and Rebecca Poulson)

Corneal Dermoids in a White-tailed Deer

During late August 2020, a private citizen in Knoxville, Tennessee reported a white-tailed deer buck that was circling, bleeding, and lacked an appropriate fear response to humans. The yearling buck subsequently was dispatched and Tennessee Wildlife Resources Agency (TWRA) personnel performed a field necropsy. Strikingly, TWRA staff noted that hair appeared to be growing from the surface of both eyes and submitted the head and selected fresh tissues to SCWDS for diagnostic examination.



Figure 3. Corneal dermoid covering the surface of the eye of a white-tailed deer.

The growths were densely haired and completely covered the surface (cornea) of both eyes, presumably drastically reducing visual capacity (Figure). Microscopic examination of the haired masses revealed they consisted of haired skin,

consistent with “corneal dermoids.” The internal structures of the eye were intact and no microscopic abnormalities were noted in the eyes or brain. Additionally, laboratory tests for hemorrhagic disease (HD) and chronic wasting disease (CWD) were conducted on the submitted tissues. In addition to the corneal dermoids, epizootic hemorrhagic disease virus (EHDV) serotype 2 was isolated from the lung. Thus, hemorrhagic disease was the presumed cause of the bleeding observed by the private citizen. It is not clear whether this buck’s abnormal behavior was attributed primarily to HD, corneal dermoids, or a combination. The prion that causes CWD was not detected in the retropharyngeal lymph nodes.

Dermoids are a type of choristoma, which is defined as normal tissue in an abnormal location. Accordingly, dermoids are characterized by skin-like tissue occurring on the body in a location other than the dermis (skin). Corneal dermoids, as in the case of this deer, often contain elements of normal skin, including hair follicles, sweat glands, collagen, and fat. The masses generally are benign (non-invasive) and are congenital, likely resulting from an embryonal developmental defect. Dermoids over the eyes likely obscure vision and disrupt an animal’s ability to forage, engage in normal social interactions, evade predation, and avoid hazards. In the present case, the age of the buck suggests that it was able to adapt and survive with this condition but ultimately succumbed to hemorrhagic disease. Frankly, it is impressive the young buck was able to survive as long as he did.

Dermoids have been reported in numerous domestic animal species, most commonly the dog and cow, but have rarely been reported in deer. In some domestic animal species and breeds, dermoids are presumed to be an inherited trait. They can affect just one eye, but often affect both eyes in cows. In contrast to the present case involving both eyes, the only previous diagnosis by SCWDS of dermoids in a white-tailed deer affected only one eye (LaDouceur et al. 2012. *Journal of Wildlife Diseases*, 48(3);826-828). Aside from obscured vision, these masses do not pose a health threat, either at the individual or population level.

SCWDS would like to thank Sterling Daniels and others at the TWRA for submission of this case showing a rare and interesting condition. (Prepared by Michelle Willis and Nicole Nemeth)

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