SCWDS BRIEFS

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Highly Pathogenic Influenza in Europe- An Update

Highly pathogenic (HP) H5 influenza A viruses (IAV) of the A/Goose/Guangdong/1/1996 lineage (GsGD-H5) have not disappeared, and from a wild bird health perspective may be more relevant than ever. We have been fortunate in North America to have experienced only one incursion of a GsGD-H5 (2014/2015). That outbreak not only resulted in waterfowl and raptor mortality but spilled over into domestic poultry and was responsible for the most expensive foreign animal disease response in US history. The virus was most likely introduced to North America through normal wild bird migration from Asia and was successfully eradicated in poultry. Luckily it did not become established in our wild bird populations, but questions related to the continued threat and potential impact of future introductions persist. For example, do we have the capabilities to detect a GsGD-H5 IAV in wild birds before we have a spillover into poultry? Can we effectively prevent a repeat of the 2014/2015 poultry outbreak if such a virus is present or established in wild birds? Could an HP H5 IAV become endemic in North American wild birds, and if so, would this represent a new and significant wildlife disease? We obviously cannot answer these questions at this time, but recent information from Europe should provide a warning to seriously consider such outcomes.

Verhagen et al. (2021) documented at least 10 incursions of HP H5 IAV in Europe between 2005 and 2020 that have resulted in significant domestic and wild bird mortalities affecting most of the continent. These incursions have been associated with different lineages of GsGD-H5 IAV and numerous genetic reassortants have been detected; these include H5N1, H5N2, H5N3, H5N4, H5N5, H5N6, and H5N8. Since 2014, and every year since, GsGD-H5 outbreaks have been associated with viruses of the 2.3.4.4 lineage and most of the genetic reassortants detected between 2005 and 2020 have been associated with this lineage. The 2014/2015 outbreak in North America was also caused by a GsGD-H5 IAV in the 2.3.4.4 lineage and as in Europe, spread rapidly within the US with migrating waterfowl. In that outbreak, genetic reassortment also was evident with the detection of H5N1, H5N2, and H5N8.

Wild bird mortality associated with GsGD-H5 IAV in Europe has been documented in at least 13 orders and 80 species. Similar with the epidemiology of low pathogenic IAV, most of these infections have been associated with species that utilize aquatic habitats such as those in the Anseriformes (duck, geese, and swans) and Charadriiformes (gulls and shorebirds). However, infections also have been documented in numerous raptor species. The diversity of species from which GsGD-H5 IAV have been detected in Europe mirrors observations made in the US during 2014/2015. This most likely corresponds to predominant IAV transmission mechanisms, with Anseriformes, Charadriiformes, and other water birds vulnerable to water-borne transmission and raptor species and other land birds vulnerable to infection through predation and scavenging on infected birds or carcasses. However, it is clear from the list of affected birds that a wide diversity of avian species are susceptible. Based on this broad susceptibility, it is possible that species attributes affecting transmission may be the most important factor limiting or enhancing the probability of infection and spread.

The current situation in Europe is not encouraging and has been described in detail in two scientific reports of the European Food and

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Safety Authority. In October and November 2020, there were 302 detections of HP H5 IAV in Belgium, Denmark, France, Germany, Ireland, the Netherlands, Sweden, and the United Kingdom. Of these, 281 were from wild birds. Most of the wild bird detections were associated with Barnacle and Greylag geese, Eurasian wigeon, mallard, and common buzzard. From December 2020 to February 2021, there were 1,022 cases; most of these detections were observed in poultry (*n*=592) but positive wild birds (*n*=421) continued to be reported. Infections were confirmed in 25 European Union countries as well as the United Kingdom. At least 48 wild bird species were reported positive in this latest report, with the top five including Barnacle goose, Mute swan, Greylag goose, Whooper swan, and Red knot. Considering the population status of many shorebird species in North America, detected GsGD-H5 infections in Red knots is particularly concerning. Because many of these detections came from dead birds and the susceptibility to the GsGD-H5 IAV is highly variable depending on both host species and specific virus lineage or strain, it is not possible to estimate the probability of infection in any one species. There have also been no estimates to date of the extent of wild bird mortality associated with this current outbreak. Although there is limited geographic surveillance coverage of wild birds globally, the overall distribution of recent clade 2.3.4.4 GsGD-H5 detections currently ranges from western Europe to eastern Asia (Korea and Japan).

The current viruses circulating in Europe are specifically classified as lineage 2.3.4.4b. Viruses in this lineage have not previously been associated with human infections in Europe but this changed this year with seven human cases (H5N8) in Russia associated with poultry workers. Fortunately, disease in these human cases was mild or inapparent. Additionally, there are no genetic indications that these viruses are increasing their potential to infect humans and at present the risk of infection is considered very low. Viruses of the GsGD-H5 lineages, however, have a history of human infection and potential severe disease so the need to maintain vigilance related to public health remains.

The final paragraph of the Verhagen et al. manuscript ends with “Ultimately, the most expedient ways to address the global One Health challenge posed by avian influenza is to effectively control poultry disease and support safe and sustainable food production, which in turn would reduce both the threat to wildlife and the zoonotic risk to humans in the long run.” The current status of wild bird surveillance and research in North America needs to be evaluated for the potential to provide early detection, guide and develop prevention and potential mitigation strategies, fully understand risk, identify the mechanisms that facilitate intercontinental movement of GsGD-H5 viruses with wild birds, and to better understand the potential for these viruses to persist or be maintained in these populations. Historically, research and surveillance related to IAV in wild birds has been extremely difficult to sustain except in times when an immediate response to an outbreak is needed. SCWDS has been very fortunate in this regard with a collection of funding from 1999 to present from USDA, CDC, and NIH through the Centers of Excellence for Influenza Research and Surveillance. Although significant progress has been made by us and others in understanding the natural history of IAV in wild bird reservoirs and the wild/domestic bird interface, there is more work to be done. As the current European situation highlights, there is a need for sustained rather than reactive vigilance in North America and this is not the time for complacency.

For more information:

Verhagen JH, et al. Highly Pathogenic Avian Influenza Viruses at the Wild-Domestic Bird Interface in Europe: Future Directions for Research and Surveillance. *Viruses*. 2021 Jan 30;13(2):212. doi: 10.3390/v13020212.

European Food Safety Authority; Adlhoch C, et al. Avian influenza overview December 2020 - February 2021. *EFSA J.* 2021 Mar 3;19(3):e06497. doi: 10.2903/j.efsa.2021.6497.

European Food Safety Authority; Adlhoch C, et al. Avian influenza overview update on 19 November 2020, EU/EEA and the UK. *EFSA J.* 2020 18(11): 6341, doi: 10.2903/j.efsa.2020.6341.

(Prepared by Becky Poulson and Dave Stallknecht)

Salmonellosis in Songbirds in North America

Beginning in January 2021 and continuing through March, a *Salmonella* spp. outbreak was

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identified in songbirds in Canada and across much of the United States, extending from the western United States to the Southeast. At SCWDS, cases have been received from Alabama, Georgia, Kentucky, Louisiana, Missouri, North Carolina, and West Virginia. The primary species received have been pine siskins (*Spinus pinus)* and American goldfinches (*Spinus tristis*) and most birds have been found sick or dead near birdfeeders in residential areas. Postmortem findings in the birds submitted to the SCWDS Research and Diagnostic Service have primarily consisted of caseous nodules in the esophagus and crop (Fig. 1) or small foci in the liver, all corresponding to areas of inflammation and necrosis (i.e., cell death) with variable numbers of bacteria. *Salmonella enterica* subsp. *enterica* serovar Typhimurium has been confirmed in all but one case, and testing is ongoing in numerous, additional, suspect salmonellosis cases.

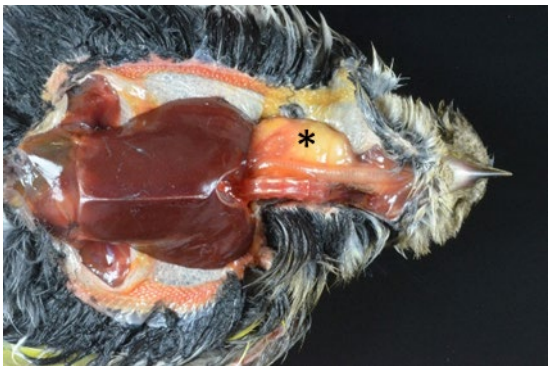

Figure 1. Pine siskin with esophageal and crop granulomas due to *Salmonella enterica* subsp. *enterica* serovar Typhimurium.

Seasonal salmonellosis mortality events in songbirds have become increasingly common in the US and some consider it to be an emergent disease potentially associated with human activity, such as the use of backyard birdfeeders. In 2004, the National Wildlife Health Center noted a 12% annual increase in the proportional mortality of *Salmonella*-related avian mortality events over a 20 year period. The overall effects of salmonellosis outbreaks on songbird populations remain unknown. It has been proposed that the current salmonellosis event may be driven by a pine siskin and finch irruption, a naturally occurring mass-migration associated with fluctuations in the availability of food resources, which the National Audubon Society reports to be one of the largest in recorded history. Neither species has been confirmed as a reservoir, but their aggressive flocking behavior (moving from feeder to feeder) facilitates the transmission of *Salmonella*.

Salmonella Typhimurium is the most commonly reported *Salmonella* species in avian salmonellosis outbreaks, as was observed in the cases diagnosed at SCWDS. The bacteria can normally inhabit the gastrointestinal tract of many species, including birds and mammals, without clinical disease and is transmitted to other animals via ingestion of food and water contaminated with bacteria-laden feces. A number of factors, such as host species, stressors, immune function, or age can predispose birds to clinical disease. Disease development can also be related to the bacterial strain and serovar virulence. Clinically, affected birds may have fluffed feathers, swollen eyelids, lethargy, weakness, or loss of coordination and may be emaciated in cases of prolonged disease. In many cases, the disease course is rapid and animals may die suddenly during feeding. Salmonellosis outbreaks tend to occur in the winter months, when birds are most reliant on backyard feeders and physiologically stressed. Common postmortem findings include variably sized abscesses and/or areas of pallor (necrosis) in the liver, spleen, and esophageal and crop mucosa. In acute (i.e., rapid) disease, animals may die before lesions develop.

Prevention of bacterial transmission is the best method of disease control. Outbreaks are commonly associated with high-density congregations of birds, including backyard birdfeeders. Birdfeeders attract unnatural, multi-species aggregations of wild birds (and other wildlife), creating an opportunity for pathogen transmission. Feeding wildlife should be discouraged but when birdfeeders are used, sound practices are critical to decreasing disease risk. Feeders that are designed to discourage birds perching (and defecating) over food should be used (e.g. “tube feeders” vs “platform” feeders). Feeders should be routinely cleaned with soap and water and sanitized with a 10% bleach solution (9 parts water and 1 part bleach) using appropriate protective equipment (gloves and mask) and allowed to dry before refilling. Removing spilled feed under feeders is also important. During outbreaks, feeders should be removed and feeding discontinued for at least 10-14 days to discourage congregation of birds and limit fecal contamination of the area.

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Salmonella species can pose a significant zoonotic (i.e., animal-to-human disease transmission) risk, especially to immunocompromised individuals. The Centers for Disease Control and Prevention (CDC) is currently investigating an ongoing outbreak of salmonellosis (caused by *Salmonella* Typhimurium infection) in people in eight states and has linked some of these infections to contact with infected wild birds or contaminated feeders (https://www.cdc.gov/salmonella/typhimurium-04-21/index.html). People infected with *Salmonella* may experience diarrhea, dehydration, fever, and stomach cramps. While most infected humans recover without intervention, the current disease outbreak has been linked to multiple hospitalizations. Some previous human disease outbreaks have also been linked to both songbirds and backyard poultry. Risk of disease transmission can be reduced with basic sanitary measures, including hand washing after handling carcasses and contaminated bird feed or feeders and limiting fecal contamination of surfaces, especially surfaces used for food preparation.

SCWDS thanks our submitters from Alabama, Georgia, Kentucky, Louisiana, Missouri, North Carolina, and, West Virginia. Laboratory tests were performed at the Athens Veterinary Diagnostic Laboratory with serovar confirmation conducted at the National Veterinary Services Laboratories in Ames, Iowa. (Prepared by Caitlin Burrell and Nicole Nemeth).

Anticoagulant Rodenticides in Eagles

In the January 2017 issue of the SCWDS BRIEFS, we highlighted a case of anticoagulant rodenticide (AR) toxicosis in a bald eagle (BAEA; *Haeliaeetus leucocephalus*) from Collier County, Florida submitted by the Florida Fish and Wildlife Conservation Commission. At the time of that article, we were three years into a five-year (2014-2018) collaborative research project to 1) determine the types of AR compounds that bald eagles and golden eagles (GOEA; *Aquila chrysaetos*) are exposed to in the United States, and 2) better define the extent of AR toxicosis in these species. We recently published the results of this study in PLoS ONE (Niedringhaus et al. PLoS ONE 16(4):e0246134, https://doi.org/10.1371/journal.pone.0246134).

Our approach to this study tapped into the machinery of the SCWDS Research & Diagnostic Service, which receives eagle carcasses for postmortem examination submitted by wildlife professionals from SCWDS state and federal partner agencies. From 2014 through 2018, SCWDS received 303 eagles (247 BAEA, 56 GOEA) for postmortem examination. The GOEA were primarily from the western US, whereas BAEA submissions were primarily from the eastern US. A complete postmortem examination was performed on each bird to determine cause of death. Eagles with gross or microscopic evidence of a bleeding disorder, along with a subset of remaining birds, were tested for AR exposure. Of the 303 eagles examined, livers from 116 BAEA and 17 GOEA were tested for ARs at the California Animal Health and Food Safety Laboratory (University of California - Davis), a key collaborator on the project. The prevalence of AR exposure (i.e., detectable levels but not associated with mortality) in eagles was high; ARs were detected in 109 (82%) eagles, including 96 (83%) BAEA and 13 (77%) GOEA. Unfortunately, the high prevalence of AR exposure observed in this study is consistent with findings in numerous other raptor species in North America and Europe. However, the impacts of AR exposure on eagles are more complicated to determine. Anticoagulant rodenticide toxicosis was determined to be the cause of mortality in 12 (4%) of the 303 eagles examined, including 11 bald eagles and 1 golden eagle. The 12 eagles with AR toxicosis had detectable levels of one or more second generation anticoagulant rodenticide (SGAR) compounds (i.e., brodifacoum, bromadiolone, difethialone, difenacoum) and seven of these birds (6 BAEA, 1 GOEA) had multiple SGARs detected. Our cases of AR toxicosis may be an underestimate because of biases and limitations associated with the passive detection of sick and dead wildlife, the lack of definitive, species-specific thresholds for toxicosis in wildlife, and the challenges of diagnosing a blood clotting disorder in the face of traumatic injuries. Further, the potential impacts of sublethal AR exposure in eagles remains unclear.

First generation anticoagulant rodenticides (FGARs) and SGARs interfere with the activation of vitamin K-dependent clotting factors in the liver, which can result in fatal hemorrhage following minor trauma or exertion during routine

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activities. Compared with FGARs, SGARs have a longer half-life in the tissues and a lower LD50, resulting in greater potential for intoxication after a single ingestion. These properties make SGARs more efficient at killing target rodent species, but they also increase the risk of non-target wildlife intoxication through both primary and secondary exposures. In our study, brodifacoum and bromadiolone, both SGARs, were the most commonly detected ARs among eagles tested. In fact, 81% of all eagles tested and 100% of eagles diagnosed with AR toxicosis had detectable levels of brodifacoum. In 2008, the United States Environmental Protection Agency (US EPA) published a final risk mitigation decision on ten rodenticide compounds aimed, in part, to reduce wildlife exposures to SGARs. This, and subsequent actions by the EPA, placed limits on the sale and distribution of products containing SGARs to general consumers and placed regulations on products for use by pest management professionals during structural pest control activities or for agricultural applications. Despite these risk mitigation actions, AR exposure continues to be a common finding in numerous wildlife taxa, as our study clearly demonstrates. The factors contributing to the high AR exposure prevalence in eagles in our study are unknown, but likely relate to the combined use of SGARs. This may be caused by the purchase and stock-piling of SGAR compounds prior to the restrictions, or the failure of the restrictions to reduce the risk of exposure since eagles may be secondarily exposed to AR compounds through currently legal applications. The frequent detection of SGARs in the eagles we examined likely reflects their popularity as a rodenticide and their tendency to persist longer in tissues. In addition to eagles and other raptors, predatory wildlife in general are at continual risk for AR toxicosis, as is evident in a variety of mammalian wildlife (e.g., black bear, bobcat, raccoon, eastern gray squirrel) evaluated by the SCWDS Research & Diagnostic Service.

This study highlights the need for future research to understand the sublethal impacts of AR exposure in eagles and other wildlife and to identify the pathways of AR exposure in eagles, both of which may help inform policy and regulatory actions to mitigate AR exposure risk. We are grateful to our many collaborators on the project and to the many state and federal wildlife agency biologists and veterinarians who submitted carcasses to SCWDS for diagnostic investigation. (Prepared by Mark Ruder and Nicole Nemeth)

Passive Immunity May Protect Deer Fawns Against EHDV

The clinical presentation of hemorrhagic disease (HD) can vary significantly in white-tailed deer, ranging from subclinical infection to acute mortality. Although multiple factors contribute to this variation, previous experiments conducted at SCWDS have shown that EHDV infection provides surviving deer with partial to full protection against reinfection. Further, we have previously demonstrated that maternally derived antibodies against EHDV persist in fawns for 17-18 weeks after birth, but the degree of protection provided by antibodies was unclear until recently.

In a recent SCWDS study, we investigated whether naturally acquired maternal antibodies to EHDV-2 could protect white-tailed deer fawns against infection and clinical disease following experimental challenge with EHDV-2. This work was recently published in the *Journal of Wildlife Diseases* (https://doi.org/10.7589/JWD-D-20-00001).

In the summer of 2016, an EHDV-2 outbreak occurred in the captive white-tailed deer research herd at the Warnell School of Forestry and Natural Resources Whitehall Deer Research Facility. Following the outbreak, seropositive and seronegative does in the herd were identified based on presence or absence of measurable EHDV-2 antibodies. The following spring, fawns born from seropositive and seronegative does were used for a challenge study with EHDV-2. The fawns remained with does for the first 48-72 hours after birth to allow for colostrum ingestion and subsequent maternal transfer of antibodies. Fawns were then pulled from does and bottle fed until 27 to 47 days of age. Serologic testing confirmed that there were six seropositive (i.e., maternal antibody-positive) and four seronegative (i.e., maternal antibody-negative) fawns in the group, based on respective presence or absence of EHDV-2 specific maternal antibodies in the blood of the fawns. Fawns were experimentally infected with EHDV-2 via subcutaneous and intradermal inoculation, and clinical response (e.g., body temperature and clinical signs), viremia and blood antibody titers were monitored for 14 days.

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The data revealed marked contrasts in both viremia profiles and clinical responses to EHDV-2 infection between seropositive and seronegative fawns. All four of the seronegative fawns displayed a combination of mild to moderate clinical signs of HD such as transient elevations in body temperature, reddened skin, and mild lethargy; however, more severe signs such as oral erosion and respiratory distress were absent. All of the seronegative fawns also had measurable viremia titers, and peak viremia titers were significantly higher than those measured in seropositive fawns, of which only two of six had measurable viremia. The clinical response was comparatively mild in the seropositive fawns, consisting only of transient elevations in body temperature in two of six fawns. Fourteen days after infection, we were able to isolate EHDV-2 virus from spleen samples from all of the fawns that had a measurable viremia.

Overall, these findings show that the presence of even a low level of maternal antibodies protects white-tailed deer fawns against clinical EHDV-2 and also reduces the level and duration of associated viremia, suggesting that passive immunity is an important component of protection against HD in fawns. Considering the timing of fawning (i.e., late spring to early summer) and HD (i.e., late summer and early fall) seasons, the presence of maternal antibodies may provide partial to full protection against infection for white-tailed deer fawns during their first HD transmission season. Whether passive protection also applies to heterologous EHDV serotypes, such as EHDV-1 or EHDV-6 or even the BTV serogroup, remains unclear. (Prepared by Natalie Stilwell and Mark Ruder)

Trauma, Lead Toxicosis, and Emaciation in an American Alligator

An adult, female American alligator (*Alligator mississippiensis*) was captured for relocation and marked with a passive integrated transponder (PIT) tag in August of 2017 in North Carolina, at which time the left eye was noticeably sunken. In December of 2018, this alligator was found in a small ditch between a busy highway and a large open field and subsequently was relocated to a less vulnerable site and was affixed with a GPS transmitter. During this capture event, the alligator was in relatively poor condition, lethargic, and both third eyelids appeared abnormal. GPS tracking data revealed that the alligator moved very little over the next two years, yet enough that biologists were able to verify that it was still alive. To retrieve and potentially replace the transmitter at the end of its battery life, this alligator was subsequently located and captured in October of 2020. When biologists determined that its overall condition had declined to such an extent that recovery seemed unlikely, euthanasia was elected. The carcass was submitted to SCWDS by the North Carolina Wildlife Resources Commission for postmortem evaluation.

On necropsy, the alligator was in poor nutritional condition, and the stomach was distended by a deflated basketball and few crayfish. An arrow shaft was embedded within the nasal passages and between the eyes and was surrounded by abundant, thick, pale tan material (exudate) and fibrosis (scarring) (Figures A-C). In addition, numerous nodules (granulomas) were scattered in connective tissue overlying internal organs (e.g., lung and liver) and also within the lung. Additionally, several collapsed (degenerated) eggs were within the reproductive tract. The fibrous tissue surrounding the arrow shaft indicated lesion chronicity, and the embedded fragmented shaft was deemed the cause of the apparent eye abnormalities noted during the previous capture events. The associated vision impairment likely contributed to diminished foraging ability and may have resulted in ingestion of inappropriate items, such as the basketball, which further inhibited its ability to acquire adequate nutrition. The granulomas throughout the body were composed of walled off, broken down blood components, consistent with internal hemorrhage due to previous traumatic insult. Based on the initial observation of the sunken eyes in this alligator followed by a 3-year decline in its condition, including malnutrition and consumption of inappropriate objects, the head trauma resulting from the embedded arrow shaft likely precipitated its slow demise.

A heavy metal (toxicology) screen performed on a sample of liver by the California Animal Health and Food Safety Laboratories in Davis, California, revealed elevated liver and iron levels, suggestive of lead toxicosis. Iron is often elevated in conjunction with high lead levels and with starvation. Although liver lead concentrations that correspond to lead intoxication are not well

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characterized for reptiles, the elevated iron level suggests secondary effects on the bone marrow, likely corresponding to the declining health status of this alligator.

Lead toxicosis is most commonly detected in dabbling waterfowl species, diving birds, and scavengers, although it has been detected in reptiles, including captive and free-ranging alligators. Birds, and presumably reptiles, most often become exposed to lead via ingestion, after which it is absorbed into the bloodstream, resulting in multiorgan and systemic effects, including kidney failure, bone marrow suppression, and neurologic signs. Toxic levels of lead can range from acute death to prolonged (chronic) disease, which often is linked with immunosuppression, as well as starvation, based on the inability to forage properly. Lead is not metabolized and accumulates over time as an animal ingests more lead products. Elevated lead levels have been reported in farmed alligators fed meat contaminated by lead bullets and have been implicated in contributing to reproductive failure in these alligators. The lead toxicosis in the current case likely contributed to its overall poor health.

Lead is also toxic to humans and domestic animals at certain levels, and care should be taken when handling possible lead contaminated products. Historically, humans have been exposed to lead associated with environmental and household sources, such as lead-based paint, water pipes, gasoline, and children's toys. Most exposures in domestic animals, particularly livestock, occur due to exposure to old batteries, lead-based paint, and used petroleum products. SCWDS thanks the North Carolina Wildlife Resources Commission for submission of this interesting case. (Prepared by Melanie Kunkel, Alicia Davis of the North Carolina Wildlife Resources Commission, and Nicole Nemeth).

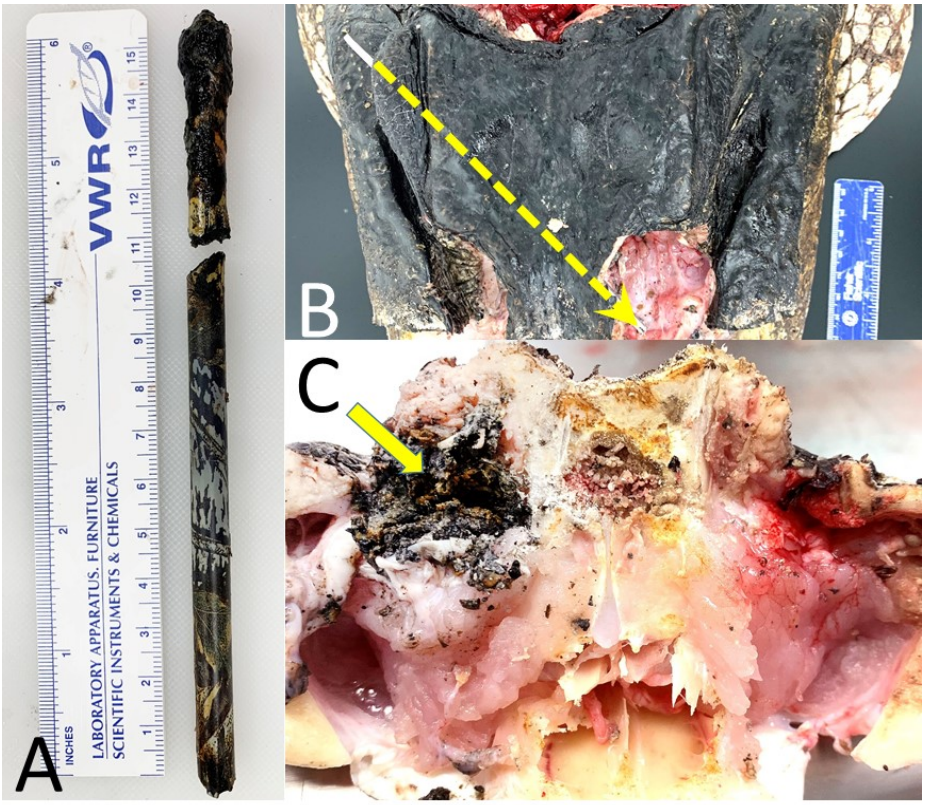

Figure A: Broken arrow shaft removed from the head.

Figure B: Alligator head showing positioning of the arrow shaft (dashed yellow line) extending from the right ear diagonally through the nasal passages to behind the left eye.

Figure C: Cross section of the back of the muzzle showing swelling, excessive tissue buildup, and discoloration where the arrow was embedded.

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