Avian Vacuolar Myelinopathy: Unraveling the Mystery

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Abstract: Avian vacuolar myelinopathy (AVM), a disease of unknown etiology, was first diagnosed in 1994 (Thomas et al. 1998) as the cause of morbidity and death of American coots *(Fulica americana)* and bald eagles *(Haliaeetus leucocephalus)* in the southeastern United States. The disease, now confirmed on 10 lakes in four southeastern states, also has been found in mallards *(Anas platyrhynchos)*, ring-necked ducks *(Aythya collaris)*, bufflehead *(Bucephala albeola)*,one great horned owl *(Bubo virginianus)*, and a killdeer *(Charadrius vociferous)*. Histological examination of the central nervous system (CNS) shows a striking diffuse, spongy degeneration of the white matter, especially in the optic tectum and cerebellum. However, assessments of epidemiology, histopathology, and environmental chemistry/toxicology have failed to determine the cause. We investigated climatic variables in relation to occurrence and duration of the disease, changes in blood chemistry of affected birds, and potential sources of exposure. The disease occurred in sentinel mallards released on Lake Surf, North Carolina, in November, but not those released on the lake in late July or early January. A decrease in water temperature and turbidity and an increase in water pH were evident during the time when the disease was evident. Serum chemistry from AVM-affected sentinel mallards and wild coots did not indicate any particular organ system dysfunction. Food habit studies suggest that mallards and coots fed primarily on *Hydrilla verticillata*. A variety of cyanobacterial algal species also were found in two water samples collected in December 2000, including several species known to produce toxins. We concluded that algal species provide the most likely explanation for the cyclic, ephemeral nature of the AVM disease agent, but diagnosis of algal toxicosis is difficult.

Key words: AVM, avian vacuolar myelinopathy, disease, coots, eagles

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Avian vacuolar myelinopathy (AVM) is a disease of unknown etiology associated with morbidity and death of bald eagles and American coots (Thomas et al. 1998). First diagnosed in 1994 at DeGray Lake in Arkansas, the disease has been found at 10 lakes in four southeastern states. Nearly 100 eagles and perhaps thousands of coots have died (T.Rocke, National Wildlife Health Center[NWHC], unpub. data)

with documented deaths in other species such as mallards, ring-necked ducks, bufflehead, one great horned owl, and one killdeer. Each lake where the disease has been confirmed is a man-made reservoir, but that is the only apparent similarity. Affected birds have been found only in late fall and winter months (October–March) and the intensity of the disease varies among years and locations.

Birds affected by AVM show clinical signs that signify impairment of the central nervous system (CNS). They are uncoordinated, ataxic, and exhibit abnormal posture, weakness, and paralysis. Severely affected birds are unable to walk or fly (Larsen et al. 2002). Histology indicates distinct lesions in the CNS characterized by diffuse, spongy degeneration of the white matter. More specifically, vacuolation occurs in the myelineated tissue of the brain and spinal cord. Given the pathology of the disease and its epidemiological history, Thomas et al. (1998) believed that a toxin was responsible. However, extensive toxicological screening of tissues from affected birds have failed to identify a source of the disease.

The goal of our study was to assist in locating the source of the potential toxin or toxicant producing the AVM lesions by examining the seasonal nature of the disease and the biotic and abiotic environmental factors existing at the time of exposure.

Study Area

This study was part of a larger study on the epizootiology of AVM (Rocke et al. 2002). Our primary site for investigation was Lake Surf, also known as Wood Lake, located in the Piedmont region of southern North Carolina, approximately 104 km southwest of Raleigh. AVM was first documented there in 1997, but there are anecdotal reports of the disease from residents of the lake dating back to 1990, when dead and moribund coots were observed (T. Augspurger, U.S. Fish and Wildlife Service, pers. commun.). Lake Surf is 452 ha with an average depth of 7.5 m. There is a dam at one end and several creeks draining into it. The dominant vegetation is hydrilla *(Hydrilla verticillata)*.

Prior to 1973, the area surrounding Lake Surf was a forested wetland. Residences and golf courses contained within a 1,200-ha fenced area now surround much of the lake. Adjacent land features include forests, wetlands, horse pastures, tobacco, and vegetable farms.

We used Lake Trace, located about 24 km northwest of Lake Surf, as our control site. Lake Trace is approximately 126 ha. Like Lake Surf, the dominant vegetation is hydrilla and it is surrounded by residential housing and a golf course. The lake is a popular site for migratory waterfowl, and has no documented occurrence of AVM.

Methods

In July 2000, November 2000, and February 2001 farm-raised mallards (Whistling Wings,[1] Hanover, Illinois) were released in groups of 30–40 birds at each of

1. Mention of trade names or commercial products does not imply endorsement or recommendation by the U.S. Government.

three locations on Lake Surf to evaluate the occurrence and duration of AVM. All were uniquely identified with numbered patagial tags. We trapped a sample (N = 3–10) from each location at weekly intervals to collect blood for serum chemistry analysis. Three to five birds were euthanized by cervical dislocation, their brains and visceral organs immersed in 10% buffered formalin, and sent to the NWHC for histological examination. The remainder were released back to the lake.

Any sentinel mallards or wild birds exhibiting clinical signs of CNS impairment also were collected immediately and processed using the above protocol. In addition, gut and esophageal contents were collected from 16 affected sentinel birds, 19 coots, and 6 unaffected sentinels from Lake Trace between 18 November and 8 December 2000. Contents were rinsed through a l.0-mm mesh screen, separated, and identified.

To determine if fish were affected by AVM, we collected a variety of species in the lake using a 30-mm gill net. Brain tissue was immersed in formalin and sent to the U.S. Geological Survey National Fish Health Research Laboratory in Kearneysville, West Virginia, for examination.

Aquatic plants were hand-pulled in six 3.34-m^2 plots on 3 January 2001 to describe the aquatic vegetation available as a food source or potential vector of the disease agent. Plots were located in approximately 0.5 to 1.5 m of water and approximately 100 m apart. Collected plants were rinsed through a 1-mm screen, separated, and identified. In addition, algal samples were collected in 1-L polyethylene I-chem bottles on 5 December 2000 from two sites in approximately 1–2 m of water where affected birds were found. We sent algal samples to the North Carolina Water Resources Division for content analysis.

Data on water quality were collected every two weeks starting in November until the end of the study. A multi-parameter water quality meter (Yellowspring Instruments, Yellowspring, Ohio) was used to measure water temperature, pH, specific conductance, dissolved oxygen, turbidity, conductivity, and oxidation-reduction potential from sites at the northern, middle, and southern end of the lake. Weather data consisting of daily minimum, maximum and average temperature, precipitation, relative humidity, wind speed, and wind direction were obtained from the North Carolina State Climatology Office.

Statistical Analysis

Blood chemistry results for 13 parameters: glucose, total protein, albumin, aspartate aminotransferase (AST), cholesterol, calcium, phosphorous, sodium, potassium, chloride, globulin, creatine protein kinase (CPK), and uric acid were compared between the pre- and post-exposure AVM affected mallards (N = 21) using a paired t-test, (α = 0.05). These parameters were compared similarly for blood samples from the AVM affected coots (N = 37) and samples from healthy coots (N = 25), previously analyzed at the NWHC in 1999.

Bi-weekly means of water quality measurements were compared using 2-sample t-tests (α = 0.05) to detect differences that may either indicate presence of a disease agent or favorable conditions for disease agents. We averaged parameters for measurements taken during months when birds were found positive for AVM and

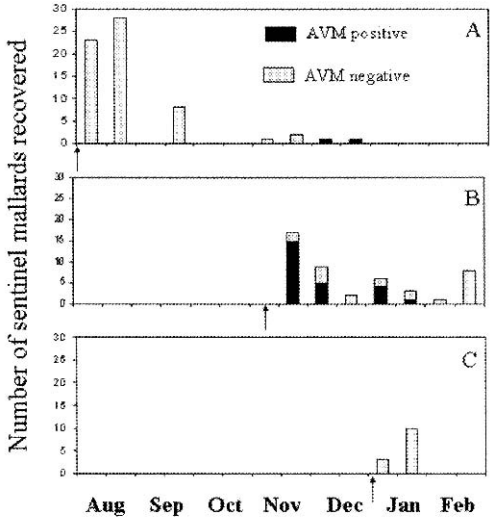

Figure 1. Occurrence of avian vacuolar myelinopathy in sentinel mallards recovered at Lake Surf, North Carolina, over two week intervals, August 2000–February 2001. Arrows indicate release dates of A) 94 birds on 31 July 2000, B) 116 birds on 8 November 2000, and C) 39 birds on 5 January 2001. Reprinted with permission from the Journal of Wildlife Diseases (Rocke et al. 2002).

compared them to measurements taken during months when the disease was not found.

Results

Of the 249 sentinel mallards released and monitored on Lake Surf between 31 July and 1 March, 124 were recaptured or found dead (Fig. 1). Twenty-seven of these, found between mid-November and late January, had brain lesions consistent with AVM. None of 25 sentinel mallards recaptured on Lake Trace tested positive for AVM.

Between November 2000 and February 2001, an estimated 1,200–1,500 coots inhabited Lake Surf. Visual estimates of the number of birds exhibiting clinical signs were made when approximately 1,500 coots were on the lake in early December. About 75% of these appeared affected by CNS disease. Fifty-eight, with clinical signs, were collected and histological examination revealed that all had AVM-type lesions. Two wild mallards and one Canada goose *(Branta canadensis)* also were collected and found positive for AVM.

Sentinel mallards with AVM had increased levels of cholesterol ($P < 0.001$), phosphorous ($P < 0.001$), and potassium ($P < 0.001$), and decreased levels of total protein ($P = 0.003$), albumin ($P = 0.005$), and globulin ($P = 0.005$) when compared with their pre-exposure blood levels. No changes were observed in levels of glucose, AST, calcium, sodium, chloride, CPK, or uric acid. Gut contents of affected sentinel mallards from Lake Surf included grit ($N = 1$), whole pieces of hydrilla ($N = 1$),

unidentifiable grass seed ($N = 6$), and Prosos millet *(Panicum miliaceum)* seeds ($N = 1$). Gut contents of control mallards from Lake Trace included corn used to bait the traps and ground invertebrate shells ($N = 6$). Gut contents of affected coots ($N = 19$) collected from Lake Surf during 17–22 November 2000, had green fluid ($N = 14$), hydrilla ($N = 7$), unidentifiable seed ($N = 8$), ground acorns ($N = 2$), and partially digested glass shrimp *(Paleomonetes sp.)* ($N = 2$).

Of 78 fish caught and sampled at Lake Surf, lake chubsucker *(Erimyzon sucetta)* were the most abundant ($N = 52$), followed by large mouth bass *(Micropterus salmoides)* ($N = 15$), black crappie *(Pomoxis nigromalculatus)* ($N = 8$), chain pickerel *(Esox niger)* ($N = 2$) and yellow bullhead *(Ameiurus natalis)* ($N = 1$). All fish appeared healthy with no obvious external lesions. Of 16 fish examined histologically, none showed vacuolar lesions in their brains.

All plots surveyed for aquatic plants contained >90% hydrilla. Two northernmost plots contained common duckweed *(Lemna perpusilla)* (~1%). One plot, centrally located on the lake, contained variable-leaf milfoil *(Myriophyllum heterophyllum)* (~2%). The southernmost plot contained Brazilian elodea *(Egeria densa)* (~4%) and brittle naiad *(Najas minor)* (~1%).

The two water samples sent for algal content analysis contained abundant amounts of *Entophysalis sp., Oedogonium sp., Tabellaria sp., Coelosphaerium sp.,* and *Melosira sp*. A number of less common species ($N = 22$) also were reported, including *Microcystis sp., Lyngbya sp.*, and *Oscillatoria sp.* These species are noted because they are known to contain or produce toxins.

Average bi-weekly temperatures were 21.8 C during March–October 2000, when the disease was not found, compared to 8.5 C from November 2000 through February 2001, when the disease was present. Weather records from 1996–2000 showed this pattern to be consistent. Monthly precipitation decreased in the months prior to the presence of the disease, while other variables including relative humidity, wind speed and direction, and standard pressure show normal fluctuation with no obvious patterns or changes.

Water was, on average, less turbid when the disease was present ($P = 0.001$) and water pH was higher ($P < 0.001$). No changes were seen in specific conductivity, dissolved oxygen, temperature, conductivity, or oxidation-reduction potential.

Discussion

Data from our sentinel mallards illustrate the seasonality of AVM. The first sentinel mallards with brain lesions indicative of AVM were found in mid-November within 7–12 days of release. No sentinel mallards with brain lesions were found prior to November and no lesions were found in birds released in early January and recaptured at the end of the month. These results are consistent with previous observations of the onset of the disease at Lake Surf and other lakes (T. Rocke, NWHC, unpub. data). The occurrence of AVM in sentinel mallards placed on Lake Surf confirm that exposure to the disease is site-specific and related to local conditions (Rocke et al. 2002). These observations and data from another related study in which

affected coots failed to transmit the disease to co-housed mallards (Larsen et al. 2002) supports the hypothesis that the causative agent of AVM is a chemical substance, most likely of natural origin (Rocke et al. 2002).

In our study, temperature declined during the time interval when AVM was present in sentinel birds and daily precipitation was significantly lower in the month prior to AVM emergence. However, more evidence is needed to determine whether there are specific patterns of temperature and rainfall associated directly with AVM. Other changes, including a decrease in relative humidity, an increase in standard pressures, and more southerly winds do not explain any particular difference in the Lake Surf environment that could lead to presence of the disease.

Water conditions indicated that changes in pH and turbidity also were associated with the presence of the disease in our sentinel birds. An increase in pH can be a normal response to an increase in photosynthesis with a consequent reduction in carbon dioxide availability. Decreasing turbidity could be caused by a number of factors including decreasing algal content, decaying vegetation, a reduction in water movement, and less precipitation (Michaud 1991). Both climatic factors and water conditions could be related to the emergence of specific toxin-producing algae or other microbes and should be monitored in future studies of AVM outbreaks.

Gut contents from the sentinel mallards and affected coots collected did not show any abnormalities. Birds fed primarily on hydrilla, which was the most available vegetation. This is similar to findings for mallards and coots on Lake Wales in Florida, where hydrilla is found (Fernald and Patton 1984). Seeds that were found in gizzards of sentinel mallards were not unusual. Grass seeds were found in birds captured while foraging on land, and millet seeds were found in birds being fed by residents of the lake. Interestingly, green fluid was found in the gut contents of 14 affected coots. This was also reported in the necropsy of affected bald eagles (Thomas et al. 1998). The green fluid may be an accumulation of bile from the gall bladder seeping into the stomach and collecting there due to lack of food digestion.

Recent laboratory experiments (Birrenkott et al. 2004, Lewis-Weis et al. 2004, Rocke et al. in press) have demonstrated that mallards, coots, and chickens fed hydrilla, from lakes with an ongoing AVM outbreak, can develop brain lesions. However, not every bird that ingested hydrilla developed lesions and no lesions developed in birds fed hydrilla from lakes without AVM outbreaks. The results of these studies suggest that the causative agent of AVM is ingested by waterbirds while consuming aquatic vegetation at affected sites. At Lake Surf, hydrilla is the dominant aquatic vegetation. However, it is not present in all AVM-affected lakes. We suspect the disease is associated with other submerged aquatic vegetation found in other affected lakes.

Based on our results from sentinel mallards at Lake Surf, we hypothesize that the AVM disease-causing agent is either seasonally accumulated by aquatic vegetation, such as hydrilla, or seasonally produced by one or more organisms associated with aquatic vegetation at affected sites that is then ingested directly by coots or other waterbirds. It is interesting that no sick or dead fish were observed during our study at Lake Surf and those examined histologically were all negative for AVM. Per-

haps they are not ingesting the same vegetation or they are not susceptible to the agent.

Algal blooms provide the most likely explanation for the cyclic and ephemeral nature of the AVM disease agent. The algal contents found in Lake Surf during the disease outbreak did contain several species known to contain or produce toxins, all of which are cyanobacteria. One of these is *Microcystis sp*., a blue-green algae that produces microcystin. Microcystin toxins are hepatotoxic and ingestion can result in death. Long term exposure may result in chronic liver damage and promote the formation of liver tumors. Two other algae identified produce neurotoxins: *Lyngbya sp*. and *Oscillatoria sp*. Saxotoxins, produced by *Lyngbya* are responsible for the syndrome known as paralytic shellfish poisoning. The saxotoxin affects acetylcholine receptors in the CNS, causing paralysis. Anatoxins are produced by *Oscillatoria*. These toxins mimic the neurotransmitter acetylcholine at its receptor, but are not cleaved by the enzyme acetylcholinesterase. This results in over-stimulation of muscles leading to paralysis and eventual death (Carmichael 1994). Diagnosis of algal toxicosis is difficult. Few methods exist to directly test for the presence of toxins and threshold levels for particular toxins on species of wildlife have not been established. Sampling of algal content, as soon as affected organisms are found, remains the most effective way to diagnose algal toxicosis due to the ephemeral nature of algal blooms (Creekmore 1999).

We had hoped that the serum chemistry of affected birds might be useful as biomarkers indicative of exposure to the causative agent of AVM, but unfortunately that was not the case. Serum chemistry was similar among samples taken from affected sentinel mallards and wild coots, but did not indicate any particular organ system dysfunction. In both species, there was evidence of dehydration from increased levels of phosphorous. Additionally, mallards showed increased levels of potassium and decreased levels of globulin. This can indicate hepatic damage, blood loss, or in this case, is most likely due to inanition or lack of food (Coles 1997). Affected birds at Lake Surf were observed either on shorelines avoiding predators by burrowing into vegetation or floating in distress on the lake. In either case, birds were not typically seen eating prior to capture. Necropsies of affected birds also showed decreased breast muscle mass and decreased subdermal fat deposits consistent with poor nutrition.

Until the causative agent of AVM is identified, the significance of the disease and potential management implications for wild birds remain unknown. Additional studies are needed to identify potential agents and to determine water and environmental conditions that are associated with the presence of AVM.

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