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ATTEMPTS TO IDENTIFY THE SOURCE OF AVIAN VACUOLAR MYELINOPATHY FOR WATERBIRDS

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ABSTRACT: Attempts were made to reproduce avian vacuolar myelinopathy (AVM) in a number of test animals in order to determine the source of the causative agent for birds and to find a suitable animal model for future studies. Submerged vegetation, plankton, invertebrates, forage fish, and sediments were collected from three lakes with ongoing outbreaks of AVM and fed to American coots (*Fulica americana*), mallard ducks and ducklings (*Anas platyrhynchos*), quail (*Coturnix japonica*), and laboratory mice either via gavage or ad libitum. Tissues from AVM-affected coots with brain lesions were fed to ducklings, kestrels (*Falco sparverius*), and American crows (*Corvus brachyrhynchos*). Two mallards that ingested one sample of *Hydrilla verticillata* along with any biotic or abiotic material associated with its external surface developed brain lesions consistent with AVM, although neither of the ducks had clinical signs of disease. Ingestion of numerous other samples of *Hydrilla* from the AVM affected lakes and a lake with no prior history of AVM, other materials (sediments, algae, fish, invertebrates, and water from affected lakes), or tissues from AVM-affected birds did not produce either clinical signs or brain lesions in any of the other test animals in our studies. These results suggest that waterbirds are most likely exposed to the causative agent of AVM while feeding on aquatic vegetation, but we do not believe the vegetation itself is the agent. We hypothesize that the causative agent of AVM might either be accumulated by aquatic vegetation, such as *Hydrilla,* or associated with biotic or abiotic material on its external surfaces. In support of that hypothesis, two coots that ingested *Hydrilla* sampled from a lake with an ongoing AVM outbreak in wild birds developed neurologic signs within 9 days (ataxia, limb weakness, and incoordination), and one of two coots that ingested *Hydrilla* collected from the same site 13 days later became sick and died within 38 days. None of these three sick coots had definitive brain lesions consistent with AVM by light microscopy, but they had no gross or histologic lesions in other tissues. It is unclear if these birds died of AVM. Perhaps they did not ingest a dose sufficient to produce brain lesions or the lesions were ultrastructural. Alternatively, it is possible that a separate neurotoxic agent is responsible for the morbidity and mortality observed in these coots.

Key words: American coot, avian vacuolar feeding trials, *Fulcia Americana, Hydrilla verticillata,* myelinopathy.

INTRODUCTION

Avian vacuolar myelinopathy (AVM) is an emerging neurologic disease of wild birds in the southeastern United States. The disease was first recognized in bald eagles (*Haliaeetus leucocephalus*) at DeGray Lake, Arkansas in 1994, and 2 yr later was confirmed in a number of American coots (*Fulica americana*) on this and another lake in Arkansas (Thomas et al., 1998). Since then, AVM has been confirmed in coots on 10 lakes in four states (Arkansas, North Carolina, South Carolina, and Georgia; Rocke et al., 2002) and also in asymptomatic birds at one reservoir in Texas (Fischer et al., 2002). Besides coots and eagles, the disease has also occurred in several species of waterfowl, including mallards (*Anas platyrhynchos*), ring-necked ducks (*Aythya collaris*), bufflehead ducks (*Bucephala albeola*), and Canada geese (*Branta canadensis*), a great-horned owl (*Bubo virginianus*), and a killdeer (*Charadrius vociferous*) (Fischer et al., 2002; Augspurger et al., 2003).

Coots clinically affected with AVM exhibit profound motor dysfunction and incoordination (Thomas et al., 1998; Larsen

et al., 2002); they are reluctant to fly, are ataxic on land, and might swim in circles or on their backs. Histologically, the disease is characterized by diffuse, spongy degeneration throughout the white matter of the central nervous system of affected birds, but not all birds with brain lesions have evident clinical signs (Rocke et al., 2002; Rocke, unpubl. data). A diagnosis of AVM requires the observation of generalized white matter vacuolation which is most prominent in the optic tectum and occurs in other regions of the brain and spinal cord when viewed with light microscopy (Thomas et al., 1998), whether the animal has apparent clinical signs or not.

Despite extensive diagnostic and field investigations, the causative agent of AVM is still unknown. Recently, the brain lesion characteristic of AVM (but not clinical signs of disease) was experimentally reproduced in red-tailed hawks (*Buteo jamaicensis*) following ingestion of tissues from AVM-affected coots, providing evidence that eagles contract the disease by consuming affected coots or ducks (Fischer et al., 2003). A subsequent experiment in which chickens were fed tissues from affected coots demonstrated that the causative agent was present in gastrointestinal (GI) contents, but not in brain, fat, kidney, liver, or muscle (Lewis-Weis et al., 2004). It has been hypothesized that the route of exposure for coots and waterbirds is also via ingestion of a contaminated food source, but previous attempts failed to reproduce the disease in mallards fed sediment, surface water, and *Hydrilla verticillata* collected during an ongoing AVM outbreak (Larsen et al., 2003).

In this paper, we summarize numerous attempts to identify the source of AVM for waterbirds by feeding test animals a variety of materials collected from various lakes during confirmed outbreaks of AVM. These simple bioassays were conducted primarily to identify the material most likely to contain the causative agent prior to more detailed analyses.

METHODS

Experimental animals

Six species were used in feeding trials in attempts to identify the source of AVM as well as to find a suitable animal model. Animals used in these trials included 30 Japanese quail (*Coturnix japonica*), 54 American coots, five American kestrels (*Falco sparverius*), 228 mallards or pekin ducks, 15 American crows (*Corvus brachyrhynchos*), and 55 laboratory mice. All animals were individually identified with wing tags, leg bands, or dye marks (mice) and held in the U.S. Geological Survey National Wildlife Health Center (NWHC, Madison, Wisconsin, USA) isolation animal facility. Japanese quail obtained from the University of Wisconsin, Poultry Science Department (Madison, Wisconsin) were housed individually in cages (76×61×41 cm). They were provided poultry feed (Purina Mills, St. Louis, Missouri, USA) and water ad libitum.

American coots of mixed sex and age were captured by night lighting at Reelfoot National Wildlife Refuge (NWR, Tennessee, USA, 36°26′N, 89°3′W) in 1997 (24 birds); Mingo NWR (Missouri, USA, 36°58′N, 90°8′W) in 2002 (28 birds); and Horicon NWR (Wisconsin, 43°30′N, 88°38′W) in 2002 (11 birds), and transported to NWHC. These sites have never had AVM to our knowledge. Several coots from each group were randomly chosen and euthanized to confirm absence of brain lesions using light microscopy. In addition, any birds that died prior to treatment were necropsied and their brains examined for lesions. Coots were housed communally on the padded floor and provided with a large swimming pool filled with water and crate in which to hide. Coots were fed ad libitum a combination of Sea Duck pellets (Purina Mills), Waterfowl Maintenance (Purina Mills), mealworms (Rainbow Mealworms, Inc., Compton, California, USA), and greens such as cilantro, parsley, and bean sprouts.

American kestrels (adults, captive-reared) were obtained from the Patuxent Wildlife Research Center (Laurel, Maryland, USA). They were housed in wire mesh flight cages (0.9× 1.8 m) provided with perches. Birds were fed 30 g meatballs or euthanized mice (Harlan Sprague Dawley, Indianapolis, Indiana, USA).

One-day-old mallard (n=133) or pekin ducklings (n=40) were purchased from Whistling Wings (Hanover, Illinois, USA) or Abendroths (Waterloo, Wisconsin, USA). Ducklings were housed communally in cages and provided with Duck Grower mash (Purina Mills) and water ad libitum. Feeding trials began when the birds were 1 wk old. Adult mallard ducks (n=55)

purchased from Whistling Wings were wing-clipped, housed communally on a padded floor in groups no larger than 25 birds, and provided access to a swimming pool. Duck Maintenance Diet (Purina Mills) and water were provided ad libitum.

Adult American crows were captured using a drop net near Wichita, Kansas (USA, 37°41′N, 97°20′W). Two were euthanized prior to treatment to confirm absence of AVM lesions. Crows were housed at NWHC, two to three per cage (76×122×175 cm), and provided perches and toys such as ping-pong balls. They were fed Science Diet Adult Canine Maintenance (Hills Pet Nutrition, Topeka, Kansas, USA), broccoli, and hard-boiled eggs.

Mice (6–8 wk old, ICR strain) were purchased from Harlan Sprague Dawley (Indianapolis, Indiana, USA) and housed in groups of four in standard laboratory mouse cages. Pelleted mouse food (PMI Nutrition International, Brentwood, Missouri, USA) and water were provided ad libitum.

Test material

Test material was collected opportunistically during AVM outbreaks from Woodlake (WL, also known as Lake Surf), North Carolina; DeGray Lake (DGL), Arkansas; and Strom Thurmond Lake (STL), on the Georgia and South Carolina border. The most extensive collections were from WL, a site where AVM has been documented nearly every year since 1997 and where we previously documented the disease in sentinel mallards and coots (Rocke et al., 2002). Test material collected from WL included lake water, submerged aquatic vegetation, sediment, plankton, small fish, and invertebrates. Samples were collected at depths <1 m about twice weekly between early October and mid-December, 2001 at three locations on the lake where waterfowl use is concentrated and affected coots have been found (Rocke et al., 2002). At the same time, mortality in wild birds was monitored and several carcasses were submitted to NWHC for diagnostic evaluation (NWHC, unpubl. data). Approximately 3 l of water and vegetation, primarily *Hydrilla* were collected in plastic freezer bags; no attempts were made to remove any of the associated epiphytes, plankton, or external debris (e.g., silt) on the vegetation. Using a shovel, about 250 ml of surface sediments (representing the top 5 cm of material) were collected. Suspended and loosely attached plankton (algae, zooplankton, phytoplankton, and other material hereafter referred to as planktonic filtrate) were obtained from areas of dense aquatic vegetation using a 153 μm mesh net. Manual net sweeps were conducted after agitation of the vegetation until about 100–200 ml of filtrate was obtained. Small fish and aquatic invertebrates were collected at one location (marina) using a seine until a sufficient sample size (approximately 100 g invertebrate material and 200 g small fish) or the sampling effort goal (two person-hr) was met. Fish collected, primarily juvenile bluegill (*Lepomis macrochirus*), represented sizes of prey items that could be consumed by coots and waterfowl and were typically <8 cm in length. Test material from DGL was collected in December 1996 and included *Elodea* (the dominant aquatic vegetation), water, duckweed (*Lemna* spp.), planktonic filtrate, and tissues from unidentified species of catfish, bass, and shad. *Hydrilla* was the only test material obtained from STL. *Hydrilla* was also collected from Harris Lake (HL; New Hill, North Carolina, USA), a site where AVM has not been diagnosed. All test materials were held on ice in the field and were stored at −20 C. Only those materials collected during confirmed AVM outbreaks in wild birds were used in the feeding studies. A portion of the material was also stored frozen for future chemical analyses if warranted by the outcome of the trial.

Prior to feeding trials, vegetation, sediment, invertebrates, and small fish were homogenized using a Waring blender. Only the muscle and GI contents were collected from larger fish (bass, shad, and catfish from DGL) and also homogenized with a Waring blender. The blended material was loaded into syringes and administered to test animals by gavage into the esophagus and/or oral cavity. In a few cases where animals would feed on the material voluntarily (e.g., mallards and *Hydrilla*), it was provided ad libitum as indicated in Table 1.

Tissues from affected coots collected at DGL, WL, and STL during ongoing AVM outbreaks and later confirmed to have brain lesions consistent with AVM were fed to mallard ducklings, crows, kestrels, and mice. Tissues from control coots collected at sites with no history of AVM and negative for brain lesions were fed to animals as a negative control. For the ducklings and mice, the tissue was homogenized in a Waring blender and administered with a syringe by gavage. For the kestrels and crows, the test material was homogenized with meatballs or a combination of meat and hard-boiled eggs to entice the animals to eat the material.

Table 1 summarizes the experiments. Amount of material fed was dependent on the size of the test animals and duration of a trial was dependent on the amount of material available from collections by field personnel. Typically, animals were fed test material until the

TABLE 1. Summary of feeding trials with various test materials collected from DeGray Lake (DGL) Arkansas, Woodlake (WL) North Carolina, and Strom Thurmond Lake (STL) South Carolina.

Test materials	Lake	Year	Test species	*n*	Dosing method	Amount	Number of days	Result
Elodea	DGL	1997	quail	4	gavage	5 g/day	7	N[7]
Elodea	DGL	1997	coots	3	ad libitum		7	ND[8]
Hydrilla	WL	1999	ducklings	20	gavage	5 ml/day	5–12	N
Hydrilla	WL	2000	mice	4	ad libitum		7	N
Hydrilla	WL	2002	coots	2	gavage	5 ml/day	9–10	2CS[9]
Hydrilla	WL	2002	coots	2	gavage	5 ml/day	38–48	1N; 1CS
Hydrilla	WL	2002	coots	2	gavage	5 ml/day	42	N
Hydrilla	WL	2002	mallards	11	gavage	10 ml/day	24	N
Hydrilla	WL	2002	mallards	20	ad libitum		23	N
Hydrilla	HL[6]	2002	coots	2	gavage	5 ml/day	29	N
Hydrilla	HL	2002	mallards	2	ad libitum		28	N
Hydrilla	STL	2002	mallards	2	gavage	10 ml/day	28	N
Hydrilla	STL	2002	mallards	4	ad libitum		23	2P[10]; 2N
Duckweed	DGL	1997	quail	4	gavage	6 g/day	7	N
Duckweed	DGL	1997	coots	3	ad libitum		7	ND
Planktonic filtrate[1]	DGL	1997	quail	4	gavage	10 ml/day	7	N
Planktonic filtrate[1]	DGL	1997	coots	3	gavage	10 ml/day	7	ND
Planktonic filtrate[1]	WL	1999	ducklings	2	gavage	3 ml/day		N
Planktonic filtrate[1]	WL	2002	coots	12	gavage	5 ml/day	18	N
Planktonic filtrate[1]	WL	2002	mallards	2	ad libitum		32	N
Sediments	WL	2002	mallards	6	gavage	10 ml/day	28	N
Water	WL	2000	mice	16	ad libitum		7	N
Fish tissues[2]	DGL	1997	quail	10	gavage	5 g/day	7	N
Fish tissues	DGL	1997	coots	3	gavage	5 g/day	7	ND
Fish	WL	1999	ducklings	16	gavage	1.5 ml/day	5–10	N
Fish[5]	WL	2002	coots	4	gavage	5 ml/day	7	N
Inverts/fish	WL	2002	mallards	2	gavage	10 ml/day	15	N
Invertebrates[4]	WL	2002	coots	4	gavage	5 ml/day	8	N
Crayfish	WL	1999	ducklings	3	gavage	1.7 ml/day	5–10	N
Shrimp	WL	1999	ducklings	16	gavage	1.5 ml/day	5–10	N
Coot muscle	DGL	1997	kestrels	2	ad libitum	20 g/day	7	ND
Coot muscle	STL	1999	ducklings	2	gavage	2.8 ml/day	7	N
Coot muscle	STL	2002	crows	2	ad libitum	20 g/day	33	N
Coot GI[3]	DGL	1997	kestrels	2	ad libitum	10 g/day	7	ND
Coot GI	DGL	1997	quail	4	gavage	6 g/day	7	N
Coot GI	STL	1999	ducklings	26	gavage	2 ml/day	5–10	N
Coot GI	STL	1999	ducklings	29	gavage	2 ml/day	7	N
Coot GI	STL	2002	crows	2	ad libitum	20 g/day	30	N
Coot kidney	STL	1999	ducklings	6	gavage	3.5 ml/day	5–10	N
Coot kidney	STL	2002	crows	2	ad libitum	20 g/day	11	N
Coot fat	STL	1999	ducklings	6	gavage	2.8 ml/day	7–11	N
Coot brain	STL	1999	ducklings	10	gavage	1.5 ml/day	5–10	N
Coot brain	STL	2000	mice	6	gavage	0.2 ml/day	5–10	N
Coot brain/fat	STL	2002	crows	2	ad libitum	20 g/day	6	N
Coot liver	STL	1999	ducklings	20	gavage	1 ml/day	5–10	N
Coot liver	STL	2000	mice	6	gavage	0.2 ml/day	5–10	N
Coot liver	STL	2002	crows	2	ad libitum	20 g/day	43	N

[1] Organisms unidentified.
[2] Fish tissues collected at DGL was muscle from catfish, muscle and intestinal contents from bass, and muscle and intestinal contents from shad.
[3] GI=gastrointestinal contents.
[4] Invertebrates collected at WL were unidentified.
[5] Fish collected at WL were primarily juvenile bluegill (*Lepomis macrochirus*).
[6] HL=Harris Lake, a lake with no known AVM outbreaks.
[7] N=negative for clinical signs and brain lesions by histology.
[8] ND=negative for clinical signs, but brains were not examined by histology.
[9] CS=clinical signs consistent with AVM.
[10] P=positive for brain lesions by histology.

amount available was nearly completely used; this was as long as 43 days in a few trials. Control animals, co-housed in the same room with the test animals, received either the negative control samples (HL *Hydrilla,* tissues from AVM negative coots) or a placebo (water or saline) for the same length of time as the test animals.

Test animals were visually evaluated at least once daily for clinical signs consistent with AVM (Larsen et al., 2002), for example, incoordination, ataxia, knuckling, or limb weakness in one or both hind limbs. Complete necropsies were performed on any animal that died or became sick and was euthanized (via CO_2 asphyxiation). Brains and other tissues were placed in 10% buffered formalin for histologic examination and tissues were collected for laboratory analyses if warranted by gross signs observed at necropsy. At the end of a trial, test and control animals were euthanized and their brains were removed for histologic examination. Brain sections were processed routinely for paraffin embedment, sectioned at 4–5 μm, and stained with hematoxylin and eosin. White matter in four regions, optic tectum, optic chiasm, medulla, and cerebellar folia, of each brain was examined by light microscopy. Our case definition for AVM included diffuse white matter vacuolation in the optic tectum and at least one other region under light microscopy as described in Thomas et al. (1998). Birds with this specific brain lesion were considered positive even in the absence of clinical signs.

RESULTS

The subsample of coots (six of 18 from Reelfoot NWR, five of 31 from Mingo NWR, and four of 11 from Horicon NWR) and crows (two of 15) examined just after capture or prior to experimentation were negative for brain lesions of AVM. Likewise all control animals (four quail, four coots from the 2002 trials, two crows, 17 ducklings, six mallard ducks, and four mice; data not shown) were also negative for AVM brain lesions.

In the earliest trials, conducted in 1997, no clinical signs were evident in any of the birds tested (quail, coots, and kestrels) and no histologic lesions were noted in the quail brains (Table 1). Unfortunately, at the time this experiment was conducted, it was not recognized that brain lesions could occur in animals in the absence of clinical signs (Fischer et al., 2003) and brains were not collected from the clinically normal coots, kestrels, or control animals.

In the subsequent trials conducted in 1999, 2000, and 2002, brains were collected from all test animals in every trial and examined by light microscopy for lesions. Clinical signs and brain lesions were not evident in the majority of animals tested (Table 1) with a few exceptions. Two of four mallards fed *Hydrilla* collected on 20 November 2001 from STL ad libitum for 24 days were found to have moderate, but distinct white matter vauolation consistent with AVM using light microscopy. Widespread vacuoles were evident in the inner white stratum of the optic lobe and optic chiasma, and although fewer, vacuoles were also present in the medulla and cerebellar folia. No clinical signs were evident in these birds. Two mallards fed *Hydrilla* from Harris Lake (HL), a site with no documented AVM outbreaks, had no signs of illness or histologic brain lesions after ingesting the vegetation for 28 days.

Two coots fed a sample of *Hydrilla* collected from one site at WL (the marina) on 6 November 2001 and used in a feeding trial in 2002 developed neurologic signs within 9 days (ataxia, limb weakness, and incoordination); one died on day 10 and the other was euthanized the following day. A subsequent sample from the same location collected 13 days later on 19 November 2001 was fed to two other coots and one of them became sick with similar neurologic signs and died on day 38. Two additional coots were fed samples of *Hydrilla* collected from the same site on 27 November 2001 for 42 days, and neither of them showed any signs of illness.

Upon necropsy of these birds, no gross lesions or abnormalities were observed, and no lesions were evident in the heart, liver, lung, kidney, or GI tract upon examination by light microscopy. Very mild, but inconclusive, vacuolation was observed in two of the three birds that showed clinical signs but not in the other four. Two

coots fed *Hydrilla* collected from HL, the lake with no documented AVM outbreaks, had no signs of illness or brain lesions after ingesting the vegetation for 29 days.

DISCUSSION

Microscopic brain lesions consistent with AVM were reproduced in two mallards only upon ingestion of a specific *Hydrilla* sample collected during an AVM outbreak at STL. Brain lesions were not evident in the majority of birds fed *Hydrilla* collected during AVM outbreaks or in birds fed samples of *Hydrilla* collected from a control site. Also, neurologic signs in three coots fed *Hydrilla* collected during a confirmed AVM outbreak at WL were indistinguishable from signs observed in sick wild birds at the time of collection. These results suggest that the causative agent of AVM might be accumulated on occasion by *Hydrilla* or similar aquatic vegetation or produced by epiphytes or other organisms associated with aquatic vegetation. We do not believe that the *Hydrilla* itself is the causative agent. We note that not all lakes with AVM positive birds contain *Hydrilla* and many lakes where *Hydrilla* is abundant have no history of AVM.

Reproduction of AVM is inconsistent. Two of four mallards fed the same sample of *Hydrilla* from STL for 23 days developed brain lesions consistent with AVM, even though clinical signs were not observed in these birds; the other two birds had no brain lesions. The agent or agents responsible for AVM may not be evenly distributed throughout the vegetation. Non-uniform distribution of the agent(s) in the field could explain the failure to reproduce the disease in mallards fed *Hydrilla* from WL in a previous study (Larsen et al., 2003) and from some of the *Hydrilla* samples collected in our experiments. Another possible explanation is that only a low dose of the causative agent(s) was present in these samples and a threshold level must be ingested before brain lesions or clinical signs are evident. Individual variations between birds in their nutritional or physiologic condition might also play a role as well as storage, transport, and freezing of samples, which might affect the agent or its potency.

Neurologic signs similar to those in wild birds with confirmed AVM were observed in three test coots fed samples of WL *Hydrilla.* Light microscopy of the brains from the three sick birds was inconclusive. No lesions were noted in other tissues from these birds either by gross or histologic examination, and no other cause of death could be determined. Furthermore, none of the coots co-housed with these birds developed similar signs of illness. We believe that these three coots died as direct result of ingestion of the Woodlake *Hydrilla* (and its associated biotic and abiotic material), but because the lesions were not conclusive by light microscopy of the brain, they did not meet our case definition for AVM. Transmission electron microscopy was attempted (data not shown), but the results were inconclusive because of formalin-fixation artifact. Perhaps myelin vacuolation was occurring primarily on an ultrastructural level or alternatively, perhaps a separate neurotoxic agent caused these deaths.

Interestingly, the occurrence of clinical signs in our test coots fed *Hydrilla* coincides with the observed morbidity of wild birds at WL in 2001. The first impaired wild coot with evident neurologic signs was found at WL on 26 October 2001, but light microscopic evaluation of its brain was inconclusive. Three other moribund coots were collected at WL on 9 November 2001, and all had brain lesions consistent with AVM. Moribund and dead coots and mallards continued to be observed from this date through mid-December. In our feeding studies, one *Hydrilla* sample collected on 6 November at WL caused neurologic signs in two of two test coots within 9 days of consumption. A second *Hydrilla* sample collected at the same site at WL on 19 November 2001 caused neurologic signs in one of two test coots within

38 days of consumption. Neurologic signs in two test coots were not evident upon ingestion of a *Hydrilla* sample collected from the same site in late November. In another study at WL conducted the year before, serial releases of sentinel mallards during the summer, fall, and winter demonstrated that exposure to the causative agent of AVM at a threshold sufficient to manifest disease (clinical signs and/or brain lesions) was seasonal and occurred over about a 2 mo period, during November and December (Rocke et al., 2002).

The experimental documentation of aquatic vegetation as a link in AVM transmission is consistent with coot feeding behavior in the wild. Coots primarily consume aquatic vascular plants and algae, with lesser amounts of grasses and other terrestrial vegetation, fish, tadpoles, crustaceans, mollusks, aquatic and terrestrial insects, and other invertebrates (Allen, 1985; Brisbin et al., 2002). They generally feed on or under shallow water where submerged or emergent macrophytes, such as *Hydrilla,* are most abundant (Brisbin et al., 2002). The range of food items for coots in the freshwater wintering grounds in North Carolina is not definitively known, but the preferred food item at WL appears to be *Hydrilla.* Coots are commonly observed dabbling and diving for this plant and consuming it in large quantities at WL, and *Hydrilla* is a common food item for coots elsewhere in the southeastern US (Brisbin et al., 2002). Coots at WL have also been observed to be stripping material, presumably algae, from the surface of *Hydrilla,* a foraging behavior noted by others (Brisbin et al., 2002). The experimental reproduction of AVM in mallards fed *Hydrilla* from STL is also relevant to the field scenario. Mallards are omnivorous and opportunistic with more reliance on aquatic vegetation in autumn and winter (Drilling et al., 2002).

In our experiments, clinical signs and brain lesions were not evident in animals that received other test materials, including sediments, fish, plankton, and invertebrates. Although further work might be necessary once the agent is identified, we believe that it is unlikely that sediments, fish, and invertebrates are commonly associated with the disease. It is interesting that the planktonic filtrate associated with WL *Hydrilla* did not result in clinical signs or brain lesions of AVM, even though it was collected at the same time and from the same location as the *Hydrilla* samples that caused clinical signs in coots. Either this material alone is not the source of AVM or the duration of feeding and/or dose was insufficient to induce disease, although the volume fed to the birds would far exceed that ingested incidentally with unwashed *Hydrilla.* Perhaps a more deliberate approach to stripping epibiotic material from the surface of *Hydrilla* should be explored.

Also, we could not reproduce the clinical signs or brain lesions in ducklings and crows that received tissues from affected birds, even those that were fed GI contents. Other investigators found brain lesions (but no clinical signs) in five of five red-tailed hawks (Fischer et al., 2003) and five of five chickens (Lewis-Weis et al., 2004) fed tissues from AVM-affected coots. These investigators determined that the agent was associated with GI contents from affected coots. Perhaps ducklings and crows are less sensitive to the agent or the amount we fed to test animals was insufficient to cause lesions. The chickens were fed 20 g of GI contents for 28 days (Lewis-Weis et al., 2004) and our crows were fed approximately 20 g for 30 days. Ducklings received 2 ml/day for 5–10 days. It is also possible that the GI content from the wild coots with AVM did not contain the toxic agent at the time of sampling or we did not feed the test material for a long enough period.

In summary, ingestion of several samples of *Hydrilla* (but not all) from lakes with ongoing outbreaks of AVM resulted in brain lesions in mallards indicative of AVM. These results support our hypothesis that the causative agent of AVM is in-

gested by waterbirds while consuming aquatic vegetation at affected sites. At WL and STL, *Hydrilla* is the dominant aquatic vegetation, however, it is not present in all AVM-affected lakes. Although we don't have definitive data, we suspect the disease is associated with other aquatic vegetation that is dominant in other affected lakes. Based on results of our previous work with sentinel mallards and coots at WL (Rocke et al., 2002), we hypothesize the 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. Also, upon ingestion of some *Hydrilla* samples collected during an AVM outbreak, several coots in our studies became sick and died with neurologic signs similar to those seen in wild birds, but lacking the characteristic brain lesions of AVM.

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LITERATURE CITED

ALLEN, A. W. 1985. Habitat suitability index models: American coot. U.S. Fish and Wildlife Service, Biological Report 82(10.115), Washington, D.C., 17 pp.

AUGSPURGER, T., J. R. FISCHER, N. J. THOMAS, L. SILEO, R. E. BRANNIAN, K. J. G. MILLER, AND T. E. ROCKE. 2003. Vacuolar myelinopathy in waterfowl from a North Carolina impoundment. Journal of Wildlife Diseases 39: 412–417.

BRISBIN, I. L., JR., H. D. PRATT, AND T. B. MOWBRAY. 2002. American coot (*Fulica americana*) and Hawaiian coot (*Fulica alai*). *In* The Birds of North America, No. 697, A. Poole and F. Gill (eds.). The Academy of Natural Sciences, Philadelphia, Pennsylvania, 44 pp.

DRILLING, N., R. TITMAN, AND F. MCKINNEY. 2002. Mallard (*Anas platyrhynchos*). *In* The Birds of North America, No. 658, A. Poole and F. Gill (eds.). The Academy of Natural Sciences, Philadelphia, Pennsylvania, 44 pp.

FISCHER, J. R., L. A. LEWIS, T. AUGSPURGER, AND T. E. ROCKE. 2002. Avian vacuolar myelinopathy: A newly recognized fatal neurological disease of eagles, waterfowl and other birds. Transactions of the North American Wildlife and Natural Resources Conference 67: 51–61.

———, ———, AND C. M. TATE. 2003. Experimental vacuolar myelinopathy in red-tailed hawks. Journal of Wildlife Diseases 39: 400–406.

LARSEN, R. S., F. B. NUTTER, T. AUGSPURGER, T. E. ROCKE, L. TOMLINSON, N. J. THOMAS, AND M. K. STOSKOPF. 2002. Clinical features of avian vacuolar myelinopathy in American coots. Journal of the American Veterinary Medical Association 221: 80–85.

———, ———, ———, ———, ———, ———, AND ———. 2003. Failure to transmit avian vacuolar myelinopathy to mallard ducks. Journal of Wildlife Diseases 39: 707–711.

LEWIS-WEIS, L. A., R. W. GERHOLD, AND J. R. FISCHER. 2004. Attempts to reproduce myelinopathy in domestic swine and chickens. Journal of Wildlife Diseases 40: 476–484.

ROCKE, T. E., N. J. THOMAS, T. AUGSPURGER, AND K. MILLER. 2002. Epizootiologic studies of avian vacuolar myelinopathy in waterbirds. Journal of Wildlife Diseases 38: 678–684.

THOMAS, N. J., C. U. METEYER, AND L. SILEO. 1998. Epizootic vacuolar myelinopathy of the central nervous system of bald eagles (*Haliaeetus leucocephalus*) and American coots (*Fulica americana*). Veterinary Pathology 35: 479–487.

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