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ATTEMPTS TO REPRODUCE VACUOLAR MYELINOPATHY IN DOMESTIC SWINE AND CHICKENS

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ABSTRACT: Avian vacuolar myelinopathy (AVM) was first recognized as a cause of bald eagle (*Haliaeetus leucocephalus*) mortality in 1994 in Arkansas (USA) and has since caused over 90 bald eagle and numerous American coot (*Fulica americana*) mortalities in five southeastern states. The cause of AVM remains undetermined but is suspected to be a biotoxin. Naturally occurring AVM has been limited to wild waterbirds, raptors, and one species of shorebird, and has been reproduced experimentally in red-tailed hawks (*Buteo jamaicensis*). In this study, chickens and swine were evaluated for susceptibility to vacuolar myelinopathy with the intent of developing animal models for research and to identify specific tissues in affected coots that contain the causative agent. Additionally, submerged, aquatic vegetation, primarily hydrilla (*Hydrilla verticillata*), and associated material collected from a reservoir during an AVM outbreak was fed to chickens in an effort to reproduce the disease. In two separate experiments, six 4-wk-old leghorn chickens and ten 5-wk-old leghorn chickens were fed coot tissues. In a third experiment, five 3-mo-old domestic swine and one red-tailed hawk, serving as a positive control, were fed coot tissues. In these experiments, treatment animals received tissues (brain, fat, intestinal tract, kidney, liver, and/or muscle) from coots with AVM lesions collected at a lake during an AVM outbreak. Negative control chickens and one pig received tissues from coots without AVM lesions that had been collected at a lake where AVM has never been documented. In a fourth experiment, eight 3-wk-old leghorn chickens were fed aquatic vegetation material. Four chickens received material from the same lake from which coots with AVM lesions were collected for the previous experiments, and four control chickens were fed material from the lake where AVM has never been documented. Blood was collected and physical and neurologic exams were conducted on animals before and once per week during the trials. All animals were sacrificed and necropsies were performed on Day 29 of feeding, with the exception of one treated chicken that was sacrificed and necropsied on Day 15 of feeding. Microscopic lesions of vacuolar myelinopathy were present in the red-tailed hawk and five chickens that received a mixture of all tissues and two chickens that received only gastrointestinal tissues of coots with AVM lesions. Three of four treated chickens in the aquatic vegetation trial developed vacuolar lesions. None of four treatment pigs or any of the negative control animals developed vacuolar lesions. Chickens are susceptible to AVM and may serve as a useful animal model for future studies. Swine may be refractory to AVM or not affected by AVM at the same dose as are chickens and red-tailed hawks. The causative agent of AVM in affected coots is associated with the gastrointestinal tissues. Furthermore, AVM can be reproduced in chickens via ingestion of aquatic vegetation and associated materials collected from a lake during an AVM outbreak. The cause of AVM is most likely present in the materials associated with submerged vegetation because the vegetation itself (hydrilla) was the same at our AVM-positive and AVM-negative sites.

Key words: American coot, avian vacuolar myelinopathy, bald eagle, chicken, hydrilla, intramyelinic edema, neurologic disease, swine.

INTRODUCTION

Avian vacuolar myelinopathy (AVM) is a neurologic disease first recognized as a cause of mortality in 1994 in bald eagles (*Haliaeetus leucocephalus*) and in 1996 in American coots (*Fulica americana*) in Arkansas (USA; Thomas et al., 1998). Augspurger et al. (2003) reported that AVM was retrospectively suspected to have caused mortality in coots as far back as 1990 in North Carolina (USA). Avian vacuolar myelinopathy has been confirmed or suspected in at least 90 bald eagle and hundreds of coot mortalities in Arkansas, South Carolina, North Carolina, and Georgia and has also been found in asymptomatic American coots at one reservoir in Texas. Avian vacuolar myelinopathy has been detected in several individuals of

other avian species from three additional taxa—Anseriformes, Strigiformes, and Charadriiformes—at one or more of the above locations (Fischer et al., 2002). The cause of AVM remains undetermined despite extensive diagnostic and research investigations; however, a natural or manmade neurotoxicant is suspected.

Gross lesions are not apparent in birds with AVM. However, there is a consistent microscopic lesion consisting of multiple vacuoles within central nervous system white matter with a predilection for the optic lobe (Thomas et al., 1998). Vacuole formation is due to splitting of the myelin laminae at the intraperiod line, characteristic of intramyelinic edema, which can be the result of a variety of causes, including acute toxicosis (Fischer et al., 2002). Agents known to cause intramyelinic edema in human beings, domestic animals, or laboratory animals include the rodenticide bromethalin (Dorman et al., 1992); hexachlorophene (Towfighi, 1980); triethyltin (Fleming et al., 1991); other manmade compounds; and toxic plants of the genera *Stypandra* (Huxtable et al., 1980) and *Heliochrysum* (Van der Lugt et al., 1996). None of these compounds or plants has been found during investigations of AVM outbreaks.

Avian vacuolar myelinopathy is characterized clinically by signs of central nervous system disease including difficulty or inability to fly, swim, or walk (Thomas et al., 1998). There appears to be a poor correlation between clinical signs and the presence of brain lesions because AVM lesions have been found in clinically normal coots collected during AVM outbreaks (Fischer, unpubl. data). Furthermore, brain lesions persisted in AVM-affected coots after the resolution of their neurologic signs in captivity (Larsen et al., 2002). Thus, AVM diagnosis must be based on the presence of microscopic lesions, rather than on clinical signs.

Avian vacuolar myelinopathy has been reproduced experimentally in red-tailed hawks (*Buteo jamaicensis*) fed tissues from AVM-affected coots (Fischer et al., 2003), demonstrating that raptors, such as bald eagles, can contract the disease through ingestion of affected coots. Aquatic vegetation material is suspected as a potential source of the AVM agent for coots because they feed heavily on aquatic vegetation (Alisauskas and Arnold, 1994). In addition to concerns regarding AVM in wild birds, questions have been raised regarding susceptibility of mammals to vacuolar myelinopathy. There have been anecdotal reports of neurologic signs in beavers (*Castor canadensis*) during AVM outbreaks, but lesions have never been confirmed (Fischer, unpubl. data). Canada geese (*Branta canadensis*) and several species of ducks (Fischer et al., 2002; Augspurger et al., 2003) have been documented with AVM, and human consumption of hunter-harvested waterfowl has raised the issue regarding potential human exposure to the AVM agent. The objectives of this study were to determine if chickens and domestic swine are susceptible to AVM and whether they could serve as animal models for future AVM research, as well as to identify specific tissues from affected coots that contain the causative agent. After associating ingestion of gastrointestinal tissues of affected coots with AVM development in chickens, we fed aquatic vegetation collected from a lake during an AVM outbreak to chickens to document this material as the potential source of the AVM agent to coots and other waterbirds.

MATERIALS AND METHODS

American coot collections

From October–April 2001–03, coots were collected from two lakes with a shotgun using number 6 steel shot. The age, sex, and weight of all coots were determined, and half of the brain was placed in 10% buffered formalin for light microscopy to confirm the presence or absence of AVM lesions. Carcasses or selected tissues (brain, fat, intestinal tract, kidney, liver, and muscle) were frozen at −18 C until AVM status was confirmed, then thawed and used in feeding trials. Determination of AVM status was based on the presence or absence of diffuse vacuolization (variable in extent) in the

white matter of the brain including the optic lobe.

American coots were collected from Clarks Hill/Strom Thurmond Lake (33°42′N, 82°20′W) on the Georgia/South Carolina border during AVM epizootics, and AVM lesions were confirmed in these birds. Negative control coots were collected from Lake Seminole, Georgia (30°48′N, 84°52′W), which has no known AVM history, and absence of AVM lesions was confirmed. In January 2003, submerged aquatic vegetation, primarily hydrilla (*Hydrilla verticillata*), was collected from both lakes and frozen at −18 C until AVM status of coots at the lakes was confirmed by microscopic examination. This material was thawed and used in the final feeding trial.

Chicken trial 1

Five male and one female 4-wk-old leghorn chickens obtained in January 2003 were used in this experiment. Chickens were housed separately to monitor food consumption. For 28 days, five treatment chickens were fed tissues from AVM-affected coots, and one control chicken was fed tissues from unaffected coots. Coot tissues fed to all animals on any given day were pooled from one to five different coots per day. To facilitate voluntary consumption, the coot tissues were rendered into a paste using an electric meat grinder. Each chicken was fed a mixture of coot tissues (total tissues $\bar{x}$[SD]=23[5] g/day, brain $\bar{x}$=1[0] g/day, fat $\bar{x}$=2[0] g/day, intestinal tract $\bar{x}$=7[2] g/day, kidney $\bar{x}$=1[0] g/day, liver $\bar{x}$=1[0] g/day, and muscle $\bar{x}$=11[4] g/day). In addition to the coot tissues, all chickens were fed $\bar{x}$=15[5] g of broiler starter scratch feed (cracked corn and wheat) per day.

Chicken trial 2

Three male and seven female 5-wk-old leghorn chickens were used in this experiment in March 2003. Chickens were housed separately to monitor food consumption. For 28 days, seven treatment chickens were fed tissues from AVM-affected coots, and three control chickens were fed tissues from unaffected coots. Of the seven treatment chickens, two chickens received only muscle tissue from affected coots; two received only liver tissue from affected coots; two received only intestines from affected coots; and one received a mixture of tissues from affected coots (brain, fat, intestines, kidney, liver, and muscle). Of the three control chickens, one received only muscle tissue from unaffected coots, one received only liver tissue from unaffected coots, and one received only intestines from unaffected coots. Coot tissues that were fed to all animals on any given day were pooled from one to five different coots. As in Chicken trial 1, the coot tissues were rendered into a paste using an electric meat grinder. All chickens were fed 20 g of their selected tissue per day. The treatment chicken getting the mixture of tissues from affected coots received brain $\bar{x}$=1(0) g/day, fat $\bar{x}$=4(0) g/day, intestinal tract $\bar{x}$=6(2) g/day, kidney $\bar{x}$=2(0) g/day, liver $\bar{x}$=1(0) g/day, and muscle $\bar{x}$=6(2) g/day. In addition to the coot tissues, all chickens were fed $\bar{x}$=60(25) g of broiler starter scratch feed per day.

Aquatic vegetation trial

Three male and five female 3-wk-old leghorn chickens were used in this experiment in July 2003. Chickens were housed separately to monitor food consumption. For 28 days, four treatment chickens were fed submerged aquatic vegetation, primarily hydrilla with associated materials, collected from Clarks Hill/Strom Thurmond Lake during a documented AVM outbreak. Four control chickens were fed submerged aquatic vegetation, primarily hydrilla with associated materials, collected from Lake Seminole, where lesions of AVM never have been found in coots. All chickens were fed 20 g of aquatic vegetation per day and $\bar{x}$=60(21) g of broiler starter scratch feed per day.

Swine trial

Five 3-mo-old male domestic swine were used in this experiment. Pigs were housed separately to monitor food consumption. Prior to the start of the trial, three pigs stopped eating and had respiratory congestion. They were treated with 1 ml of Naxcel® (ceftiofur sodium; GlaxoSmithKline, Research Triangle Park, North Carolina) via intramuscular injection per day for 4 days. Antibiotic treatment concluded 1 day prior to the feeding trial, and pigs were noticeably improved. One rehabilitated, unreleasable, adult male red-tailed hawk was obtained from the Carolina Raptor Center, Charlotte, North Carolina. This hawk was used as a known susceptible host to confirm the presence of the AVM agent during the feeding trial. This individual was initially found in Mecklenburg County, North Carolina, which is an area where AVM has never been documented and is approximately 150 km from the nearest known AVM-positive site.

For 28 days, four treatment pigs and the red-tailed hawk were fed tissues from affected coots, and one control pig was fed tissues from unaffected coots. Tissues fed to all animals on any given day were pooled from one to five different coots. Of the four treatment pigs, one

was fed only muscle tissue from affected coots ($\bar{x}$=480[143] g/day), and the other three treatment pigs each were fed a mixture of tissues from affected coots (total tissues $\bar{x}$=575[198] g/day, brain $\bar{x}$=2[0] g/day, fat $\bar{x}$=45[33] g/day, intestinal tract $\bar{x}$=338[124] g/day, kidney $\bar{x}$=12[6] g/day, liver $\bar{x}$=34[14] g/day, and muscle $\bar{x}$=144[72] g/day). In addition to the coot tissues, all pigs were fed $\bar{x}$=155[14] g of corn feed per day. The red-tailed hawk was force-fed a mixture of tissues from affected coots: total tissues $\bar{x}$=95(21) g/day, brain $\bar{x}$=2(0.8) g/day, fat $\bar{x}$=11(6) g/day, intestinal tract $\bar{x}$=30(10) g/day, kidney $\bar{x}$=4(2) g/day, liver $\bar{x}$=14(6) g/day, and muscle $\bar{x}$=34(11) g/day.

Neurologic and physical exams

Throughout the trials, all animals were observed once per day for 1 hr in the mornings for signs of neurologic disease or other health problems. Prior to the feeding trials and once a week after the feeding trials began, blood samples were collected and basic physical and neurologic exams were performed on all animals, with the exception of the hydrilla trial in which only pre- and posttrial blood samples were taken. Complete blood counts and serum chemistries (total protein, albumin, glucose, sodium, potassium, chloride, bicarbonate, anion gap, calcium, aspartate transaminase, creatine kinase, lactate dehydrogenase, cholesterol, bile acids, and uric acid) were conducted (University of Georgia, College of Veterinary Medicine, Clinical Pathology Laboratory, Athens, Georgia). For the swine trial, a neurologic examination form, adapted from Oliver et al. (1997), was developed in order to standardize the examination and the recording of observations. The red-tailed hawk and chicken neurologic exams followed protocol developed for domestic birds (Clippinger et al., 1996). All neurologic examinations included assessment of mental status, level of consciousness, posture, conscious proprioception, gait, and sensory and motor responses. Thus, animals were first observed from the outside of the stall/cage. Parameters such as mentation, posture, attitude, gait, and movement were best evaluated before the animal became excited from handling. Parameters such as conscious proprioception and motor function were evaluated further upon manual restraint. Function of cranial nerve I was determined during feeding. Function of cranial nerves II–VI was evaluated by assessing vision, menace response, pupillary light reflex, eye and pupil symmetry, and palpebral response. Function of cranial nerves V and VII was evaluated further by assessing mandible function, motor response to facial stimulation, and facial symmetry. Function of cranial nerve VIII was evaluated by assessing the animal's balance and coordination. Function of cranial nerves IX–XII was indirectly evaluated by observing the animals for evidence of dysphagia. In addition, tongue position and tone were evaluated.

Necropsy

During chicken trial 1, one treatment chicken was sacrificed on Day 15 because of clinical illness and necropsied. On Day 29 of the three trials, all animals were sedated with a ketamine/xylazine (10 mg/kg and 0.5 kg/mg, respectively; Fort Dodge Animal Health, Ft. Dodge, Iowa, USA) mixture, sacrificed with sodium pentobarbital (Beuthanasia, 1 ml/4.5 kg; Schering-Plough Animal Health Corporation, Union, New Jersey, USA), and a full necropsy was performed. Half of the brain and portions of the sciatic nerve and spinal cord were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and examined by light microscopy. Other tissues examined using light microscopy included trachea, lung, heart, liver, spleen, kidney, adrenal gland, intestine, gonad, skeletal muscle, and pancreas. Transmission electron microscopy was used to confirm microscopic lesions in one chicken from each chicken trial. Samples of optic lobe were placed in 2% glutaraldehyde, 2% paraformaldehyde, and 0.2% picric acid in a 0.1 M cacodylate buffer (pH 7.2). Following fixation, the samples were postfixed in 1% osmium tetroxide, dehydrated in a series of alcohols, stained in bloc with uranyl acetate, and embedded in epoxy resins. Sections 1-μm thick were stained with toluidine blue and examined by light microscopy to select areas with vacuolar lesions of white matter. Ultrathin sections of these areas were stained with uranyl acetate and lead citrate and examined with a JEOL Model JEM-1210 transmission electron microscope (JEOL USA, Inc., Peabody, Massachusetts, USA).

RESULTS

Chicken trial 1

From Days 10–14 of feeding, all treatment chickens exhibited increased sedentary behavior that continued until the end of the trial. From Days 12–18, during casual observation, all treatment chickens exhibited some form of ataxia, imbalance, or drooping wings and/or tail; however, only one chicken showed these signs during the complete neurologic exam performed on

Day 15. This chicken was sacrificed and necropsied on Day 15. No gross lesions were apparent upon necropsy, and the chicken appeared to be in good body condition with adequate muscle mass and some body fat. Microscopic lesions of vacuolar myelinopathy were not apparent in this chicken.

During neurologic exams performed on Days 22 and 29 of feeding, clinical signs became apparent in the remaining treatment chickens. All treatment chickens were very sedentary and would only stand for a few minutes before lying back down. All had varying degrees of ataxia, imbalance while standing or lying down, constant wing and/or tail droop, an unthrifty appearance, and a hesitant gait; several began defecating on themselves. Four of the treatment chickens had mydriasis of one or both pupils beginning at Day 22. There was some reduction in food consumption, but most had fair to good appetites even after the onset of clinical signs. Average body weight of all chickens including the control, which had increased from 160 to 302 g, began to decrease the last week of the trial. During the entire trial the control chicken remained alert and active and showed no signs of disease. All blood counts and serum chemistry results were within normal limits for chickens (Spano et al., 1987; Canadian Council on Animal Care, 1993; Bounous and Stedman, 2000) and did not differ from the negative control chickens.

At necropsy, all chickens, including the control chicken, had gross and microscopic lesions consistent with rickets, and all appeared to be in poor to fair body condition with some breast muscle atrophy and little or no body fat. Microscopically the four treatment chickens had vacuolar lesions that consisted of mild to moderate vacuolization of the white matter in the optic lobe. Two chickens also had lesions in the optic nerve; however, lesions were not apparent elsewhere in the brain, spinal cord, or sciatic nerve of any birds. Transmission electron microscopy of the optic lobe white matter of an affected chicken revealed numerous vacuoles delimited by myelin laminae that had split at the intraperiod line. Lesions of AVM were not observed in the central nervous system (CNS) of the control chicken. There were no other significant lesions apparent in sections of sciatic or other peripheral nerves, trachea, lung, heart, liver, spleen, kidney, adrenal gland, intestine, pancreas, gonad, and skeletal muscle in any of the chickens.

Chicken trial 2

All chickens appeared clinically normal and had good appetites, with the exception of one chicken receiving intestines from AVM-affected coots. This bird had anisocoria, in this particular case a rapid unilateral fluctuation between mydriasis and myosis, during neurologic exams on Days 22 and 29 of feeding. Average body weights of all chickens steadily increased every week ($\bar{x}$=278–742 g). No gross lesions were apparent at necropsy, and all chickens appeared to be in good body condition with adequate muscle mass and some body fat. All blood counts and serum chemistry results were within normal limits for chickens and did not differ from the negative control chickens.

The chicken receiving a mixture of tissues from affected coots had mild microscopic vacuolization of the white matter in the optic lobe, but lesions were not evident in other parts of the CNS. The two chickens receiving intestines from AVM-affected coots had AVM lesions that consisted of mild to moderate vacuolization of the white matter in the optic lobe, again with no other lesions evident in the CNS. Transmission electron microscopy of the optic lobe white matter of one affected chicken revealed numerous vacuoles delimited by myelin laminae that had split at the intraperiod line. Lesions of AVM were not present in the CNS of chickens receiving muscle and liver or control chickens. There were no significant lesions apparent in sections of sciatic or other pe-

ripheral nerves, trachea, lung, heart, liver, spleen, kidney, adrenal gland, intestine, pancreas, gonad, and skeletal muscle of any of the chickens.

Aquatic vegetation trial

None of the chickens demonstrated clinical signs of neurologic disease. Average body weights of all chickens steadily increased every week ($\bar{x}$=162–484 g). No gross lesions were apparent at necropsy, and all chickens appeared to be in good body condition with adequate muscle mass and some body fat. All blood counts and serum chemistry results were within normal limits for chickens and did not differ from the negative control chickens.

Three of four treatment chickens had mild microscopic vacuolization of the white matter in the optic lobe, but lesions were not evident in other parts of the CNS. Transmission electron microscopy of the optic lobe white matter of one affected chicken revealed numerous vacuoles delimited by myelin laminae that had split at the intraperiod line. Lesions of AVM were not present in the CNS of the control chickens. There were no significant lesions apparent in sections of sciatic or other peripheral nerves, trachea, lung, heart, liver, spleen, kidney, adrenal gland, intestine, pancreas, gonad, and skeletal muscle of any of the chickens.

Swine trial

None of the pigs demonstrated clinical signs of neurologic disease. All blood counts and serum chemistry results were within normal limits for swine (Tumbleson et al., 1986) and did not differ from the prefeeding or control pig values. No gross lesions were apparent upon necropsy, and all animals were in excellent body condition with adequate muscle mass and ample body fat. Significant microscopic lesions were not apparent in multiple sections of the brain (optic nerves, optic chiasma, optic tracts, hippocampus, corpus callosum, thalamus, cerebral cortex, cerebellar folia, and brainstem); sciatic nerve; spinal cord; trachea; lung; heart; liver; spleen; kidney; adrenal gland; intestine; gonad; skeletal muscle; and pancreas of any pig. However, microscopic lesions of vacuolar myelinopathy were apparent in the red-tailed hawk that had received tissues from coots with AVM. Lesions consisted of mild to moderate vacuolization of the white matter and were most prominent in the optic lobe, white matter tracts of the cerebrum, and the cerebellar folia.

DISCUSSION

Chicken trials

These studies demonstrate that chickens develop AVM lesions when orally exposed to tissues from coots with lesions, adding another species within a different order (Galliformes) to the species of birds susceptible to AVM. Chickens that developed AVM lesions were fed an average of 9% and 4% of affected coot tissues by body weight in trial 1 and trial 2, respectively. Chickens in trial 1 that developed lesions were fed slightly more affected coot tissues by body weight (5.2–15.0%, $\bar{x}$=9%) than the red-tailed hawk in the swine trial (3.6–8.8%, $\bar{x}$=6%) and red-tailed hawks in previous research (1.3–7.3%, $\bar{x}$=3%; Fischer et al., 2003). This higher exposure may account for the clinical signs that became apparent halfway through trial 1; however, the effects of rickets makes interpreting the AVM clinical signs more difficult. Rickets can cause clinical signs similar to those in this study, including reluctance to stand up and/or walk (Austic and Scott, 1984). However, some if not all of the clinical signs were compatible with and more likely caused by AVM because the control chicken also had rickets but did not show clinical signs. It is possible that the nutritionally compromised status of the chickens in trial 1 allowed clinical signs to develop sooner and with more severity compared with healthier treatment chickens in trial 2. These findings may indicate that the health status of wild birds may influence their susceptibility to the disease as well as their ability to recover. Of fur-

ther interest is the lack of lesions compatible with either AVM or rickets in the chicken with clinical signs necropsied on Day 15 of feeding. Other researchers also have noted lack of lesions in apparently clinically ill coots in their laboratory efforts to reproduce the disease (Rocke, pers. comm.). In contrast, mallard ducks (*Anas platyrhynchos*) allowed to feed/swim freely during a natural AVM outbreak in North Carolina displayed clinical signs and had moderately severe lesions as soon as 5 days postexposure (Rocke et al., 2002).

The chickens in trial 2 receiving intestines or the mixture of tissues containing intestines from AVM-affected coots developed AVM lesions, suggesting the causative agent of AVM is present in the gastrointestinal tract as a result of ingestion. Previous testing of stomach contents of AVM-affected coots and bald eagles have yielded no known toxins or toxin-producing agents (Thomas et al., 1998).

Aquatic vegetation trial

Results of this trial demonstrated lesion development in chickens following ingestion of aquatic vegetation material, primarily hydrilla, collected from a lake during an AVM outbreak. Although one treatment chicken did not develop apparent AVM lesions, this bird consistently consumed very little vegetation material and may not have been exposed to enough of the AVM agent to become affected. Development of AVM lesions in chickens receiving aquatic vegetation, as well as in chickens that receive only the gastrointestinal tissues, including their contents, strongly indicate that the AVM agent is associated with aquatic vegetation. In this particular study hydrilla was the primary aquatic vegetation used. Hydrilla is an invasive exotic plant that was first discovered in Florida in the 1960s (Blackburn and Weldon, 1969) and has since spread throughout the Southeast and other regions of the US. Although hydrilla may be involved in some way with the AVM agent, it should not be singled out as the cause. Hydrilla is not found at every location that has AVM-affected birds nor does AVM exist at every location that has hydrilla. It is possible that the causative agent is associated with submerged aquatic vegetation regardless of species, and hydrilla was simply the first species discovered to be involved.

Swine trial

The pigs in this study did not develop vacuolar lesions although they were fed approximately the same daily amount (1.8–2.1%, $\bar{x}$=2% by body weight) of tissues from AVM-affected coots known to cause AVM lesions in red-tailed hawks (1.3–7.3%, $\bar{x}$=3% by body weight; Fischer et al., 2003). The tissue/body weight dosage technique was used because the exact amounts of the causative agent of AVM contained in the tissues is unknown and likely varies from coot to coot. Fischer et al. (2003) demonstrated consistent production of AVM in red-tailed hawks when tissues from affected coots were fed at a rate of 1.3% of body weight. Due to rapid growth of the pigs and a finite amount of available coot tissues, it was difficult to maintain the 3% body weight ratio goal. The positive control red-tailed hawk received 3.6–8.8% ($\bar{x}$=6%) of its body weight in affected coots.

The additional feed and antibiotics used in the pigs are factors that may have influenced the susceptibility of swine in this study, but these were unavoidable variables. We do not feel that the antibiotics played any part in the failure to reproduce AVM because there is no evidence that AVM is of a bacterial etiology (Thomas et al., 1998). In previous research (Fischer et al., 2003), one red-tailed hawk was treated with antibiotics 5 days before being fed affected tissues and still developed AVM lesions. It is possible that pigs are refractory to AVM or not affected by AVM at the same dose as are red-tailed hawks.

Results of these trials reveal that swine may be refractory to vacuolar myelinopathy or are not as susceptible as red-tailed

hawks or chickens, and this may apply to other mammals; however, factors including dose and exposure time have not been adequately tested. This study demonstrates that chickens are susceptible to AVM and could serve as a suitable animal model for future research. The development of lesions in chickens ingesting gastrointestinal tissues from AVM-affected coots focused our attention on materials that coots were consuming, and the aquatic vegetation trial established that AVM lesions can be produced in chickens via ingestion of aquatic vegetation collected during an AVM outbreak. Investigations to identify the causative agent should focus on those materials associated with submerged aquatic vegetation in reservoirs in which AVM occurs. These materials may include other organisms that may produce or biomagnify toxicants. Additional research is needed to determine species susceptibility, the pathogenesis of the disease, the predictability of outbreaks, and the development of management options to prevent or minimize the impact on wildlife.

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