Failure to Transmit Avian Vacuolar Myelinopathy to Mallard Ducks

Authors: R. Scott Larsen, Felicia B. Nutter, Tom Augspurger, Tonie E. Rocke, Nancy J. Thomas, et. al.

Source: Journal of Wildlife Diseases, 39(3) : 707-711

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-39.3.707

Journal of Wildlife Diseases, 39(3), 2003, pp. 707–711

Failure to Transmit Avian Vacuolar Myelinopathy to Mallard Ducks

R. Scott Larsen,[1,2,5,6] Felicia B. Nutter,[1,2] Tom Augspurger,[1,3] Tonie E. Rocke,[4] Nancy J. Thomas,[4] and Michael K. Stoskopf[1,2] [1] Environmental Medicine Consortium, [2] Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, North Carolina 27606, USA; [3] United States Fish and Wildlife Service, Raleigh Field Office, P.O. Box 33726, Raleigh, North Carolina 27636, USA; [4] U.S. Geological Survey, Biological Resources Division, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, USA; [5] Current address: Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California 95616, USA; [6] Corresponding author: (email: slarsen@ucdavis.edu)

ABSTRACT: Avian vacuolar myelinopathy (AVM) is a neurologic disease that has been diagnosed in free-ranging birds in the southeastern United States. Bald eagles (*Haliaeetus leuocephalus*), American coots (*Fulica americana*), and mallards (*Anas platyrhynchos*) have been affected. Previous investigations have not determined the etiology of this disease. In November and December 2002, we attempted to induce AVM in game-farmed mallards through four, 7-day exposure trials. Mallards were housed in six groups of eight, with two of these groups serving as controls. One group was housed with AVM-affected coots; one group was tube fed daily with water from the lake where affected coots were captured; one group was tube fed daily with aquatic vegetation (*Hydrilla verticillata*) from the same lake; and another group was tube fed daily with sediment from the lake. No ducks exhibited clinical neurologic abnormalities consistent with AVM and no evidence of AVM was present at histopathologic examination of brain tissue. Although limitations in sample size, quantity of individual doses, frequency of dose administration, duration of exposure, and timing of these trials restrict the interpretation of the findings, AVM was not readily transmitted by direct contact, water, hydrilla, or sediment in this investigation.

Key words: American coot, *Anas platyrhynchos,* avian vacuolar myelinopathy, *Fulica americana,* mallard.

Avian vacuolar myelinopathy (AVM) is a neurologic disease of free-ranging birds that is characterized by profound neurologic dysfunction and debilitation (Thomas et al., 1998). The disease has been diagnosed most frequently in American coots (*Fulica americana*) and bald eagles (*Haliaeetus leucocephalus*), but it has also been observed in mallard ducks (*Anas platyrhynchos*) and other waterfowl (Rocke et al., 2002; Augspurger, unpubl. data). It is characterized by diffuse, spongy degeneration throughout the white matter of the central nervous system (Rocke et al., 2002; Thomas et al., 1998). An associated cellular inflammatory response has not been documented and attempts to isolate viruses, bacteria, and fungi have been unsuccessful (Thomas et al., 1998). No consistent lesion has been evident in other body systems and definitive diagnosis is currently based entirely on histopathologic findings (Thomas et al., 1998). While many diseases could cause neurologic impairment in eagles, coots, and other birds, no other disease with similar vacuolar lesions has been reported in a free-ranging avian population. The only reports of similar lesions in birds have described vacuolations that were experimentally induced using trimethyltin or isonicotinic acid hydrazide (Carlton and Kreutzberg, 1966; Lampert and Schochet, 1968; Fleming et al., 1991).

Extensive tissue analyses have been conducted in an attempt to detect toxic compounds; however these tests have not elucidated the etiology of AVM (Thomas et al., 1998). In late fall 2000 we conducted exposure trials to assess possible routes of disease transmission. Four methods of exposure were investigated, including direct exposure to affected birds, ingestion of water, ingestion of aquatic plants, and ingestion of sediment from a lake where affected birds were present. Forty-eight, 16 wk old, game-farmed mallard ducks were purchased from Whistling Wings, Inc.® (Hanover, Illinois, USA). They were transported to Raleigh, North Carolina, USA (35°50′N, 78°40′W) where they were housed in

7×11 m outdoor flight pens that contained 3×5 m, man-made dirt ponds. Although coots are the species most commonly found with AVM in the wild, and thus may be more susceptible to the disease, they were not selected for this investigation because they can be relatively difficult to keep in captivity and are prone to self-induced trauma (Larsen et al., 2002). Game-farmed mallard ducks were selected because similar mallards have developed clinical signs and histopathologic lesions when placed on a lake where AVM-affected coots were found (Rocke et al., 2002). The mallards (20 male, 28 female) were allowed to acclimate for 25 days prior to the exposure trials, during which time they were fed a commercial poultry diet (High Tech X-L-A, Quality Feeds Small and Special Poultry Flocks, Southern States Cooperative, Richmond, Virginia, USA). When the exposure trials began, the diet was changed to a mash of the commercial poultry diet with a top dressing of bean sprouts. Complete standardized physical examinations (Harrison and Ritchie, 1994) and neurologic evaluations (Bennett, 1994) were conducted on all ducks within 1 wk of arrival. Primary and secondary flight feathers of one wing were trimmed and colored leg bands were applied for identification. Mallards were separated into six groups of eight by random allocation with stratification for gender. Four groups were used in 7-day exposure trials and the other two groups served as controls. Mallards were visually evaluated twice daily, weighed on days 1, 4, and 7 of the 7-day trial, and received complete standardized physical examinations and neurologic evaluations on days 4 and 7. This length of exposure was chosen because game-farmed mallards had previously been documented with AVM within 6 days of being placed on a lake with AVM-affected coots (Rocke et al., 2002). All mallards were euthanized with 117–234 mg sodium pentobarbital (Beuthanasaia®-D Special, Schering Plough Animal Health Corporation, Kenilworth, New Jersey, USA) intravenously on day 7 of the exposure trials. Brains were removed and immersed in 10% neutral buffered formalin for histopathologic examination. Brain sections were routinely processed, embedded in paraffin, sectioned at 5–6 μm, and stained with hematoxylin and eosin. White matter in four regions of each brain was examined by light microscopy: optic tectum, optic chiasm, brainstem, and cerebellar folia. A bird was considered positive if diffuse white matter vacuolation was present in the optic tectum and at least one other region. Only brain tissue was examined in the exposed ducks because this has been the only tissue consistently affected in eagles, coots, and mallards (Thomas et al., 1998; Thomas, unpubl. data).

The first two transmission trials were conducted from 28 November to 4 December 2000. Direct transmission was evaluated by placing eight mallards (three male, five female) in a pen with neurologically impaired American coots from Lake Surf, North Carolina (35°14′N, 78°12′W). Twelve affected coots were introduced on day 1; 12 additional affected coots were introduced on day 4. Many of the coots died during the course of the exposure trial and ducks were housed with 5–15 (median=10) coots at any one time. All 24 coots died or were euthanized. Brains were removed, processed, and examined microscopically as described for the mallards, and all were confirmed by histopathology to be AVM-positive with widespread, bilaterally symmetrical vacuolation of the white matter of the brain.

Transmission by ingestion of water was evaluated by administering 50 ml of water from Lake Surf, once daily, using a lubricated red-rubber feeding tube inserted orally to the level of the crop of each of the second group of mallards (three male, five female). The water administered was taken from several different sites at Lake Surf over the course of the seven-day exposure trial and was stored at 4 C in cer-

tified-clean glass jars with Teflon®-lined closures, for 24–72 hr prior to administration. A third group of mallards served as a control during these trials and received only full physical and neurologic evaluations on days 4 and 7.

Additional transmission trials were conducted from 6 December to 12 December 2000. Transmission via aquatic plant ingestion was evaluated by administering hydrilla (*Hydrilla verticillata*) from several different sites at Lake Surf. Hydrilla was chosen because, at this lake, it is both the dominant submerged macrophyte and the preferred food source for American coots. The hydrilla was stored at 4 C for 24–72 hr prior to administration. Directly before administration to the mallards (four male, four female), hydrilla (80 g) was mixed at high speed in a blender with 40 ml of distilled water. Fifty ml of the resultant slurry was administered to each bird, once daily, via red-rubber tube, similar to the procedure described for the water ingestion trial.

Transmission via sediment ingestion was assessed by tube feeding mallards (three male, five female) with samples of sediment from several different sites at Lake Surf. Sediments were collected with a stainless steel Ponar® dredge, a device that collects the top 5 cm of sediment. Samples were collected in shallow water (<2 m), near shore, in areas where coots were observed feeding or resting. Sediment samples were homogenized and then stored in certified-clean glass jars with Teflon®-lined closures for 24–72 hr prior to administration. They were filtered through a metal sieve to remove large particulate matter, and then 50 ml of this fine sediment slurry was administered to each bird, once daily, using a red-rubber tube. Another group of eight ducks served as controls for these trials and received 50 ml distilled water, once daily, by red-rubber tube.

There were no abnormal physical findings in any of the ducks. The only behavioral difference noted was that ducks orally gavaged on a daily basis became progressively more aggressive and more difficult to catch. Neurologic abnormalities were not apparent. No gross abnormalities were observed at postmortem examination and no microscopic abnormalities were seen in the brain of any of the mallards.

There are many potential reasons why no disease transmission was achieved, including the possibility that the disease is not transmitted by any of the mechanisms that we investigated. In addition to aquatic vegetation, coots also feed on insects, crustaceans, mollusks, and small fish (Taylor, 1998) and it may be useful to investigate these food items as potential sources of transmission. It is also possible the exposures we administered were inadequate in duration to cause disease. Seven-day exposure times may have been inadequate under the conditions of captivity, even though AVM has been detected in game-farmed mallards within 6 days of being released on the same lake that we used as our exposure source (Rocke et al., 2002). The exposures may have been insufficient in volume or concentration; however the volumes of material administered reflected the maximum volume that could be safely administered in a single dose. Efforts to administer higher volumes resulted in regurgitation in several ducks. However, to minimize the effects of handling and stress, administration of materials was only performed once per day and it is possible that birds in the wild ingest greater than 50 ml of one or more of these items over the course of a day. Mallards in captivity have been reported to drink approximately 350 ml of water per day (Rowe and Prince, 1983). In regard to the exposure rate to affected birds, it seems unlikely that there would be a higher contact rate between mallards and coots in the wild.

The sample size we selected may have been too small to detect clinical disease. Although high numbers of coots are affected during naturally occurring outbreaks, few affected individuals of other species are typically found (Augspurger,

unpubl. data). This may reflect a lower susceptibility for other species, such as mallards, so higher numbers may need to be exposed to ensure characteristic lesions develop. However, a previous investigation suggested that the disease in sentinel mallards closely mimicked the disease in wild coots and other waterfowl (Rocke et al., 2002). The detected prevalence of AVM in sentinel mallards has been highly variable, ranging from 0–54% (Rocke et al., 2002), but the reason for this variability is undetermined and is potentially multifactorial. When vacuolar myelinopathy has been induced in captive ducks using established neurotoxins, nearly 100% of the exposed birds have developed characteristic lesions (Carlton and Kreutzberg, 1966; Lampert and Schochet, 1968; Fleming et al., 1991).

Another potential explanation for our inability to transmit disease is that we did not collect the samples for exposure trials at a time when the causative agent was present at the lake. In fall 2000, the first confirmation of AVM in wild birds at Lake Surf occurred on November 16, 2000. Samples for transmission studies were collected from that lake in late November and early December, after affected coots first appeared, but while affected coots were still visible on the lake. A published description of AVM suggested that eagles survived for only a few days after first exhibiting neurologic abnormalities (Thomas et al., 1998). However, recent research has shown that coots can survive for weeks after the first signs of neurologic dysfunction and clinical recovery may occur (Larsen et al., 2002). Furthermore, both coots and mallards may have histologic lesions, but show no signs of disease (Larsen et al., 2002; Rocke et al., 2002). This implies that free-ranging coots could be present on an affected lake for days to weeks after being exposed to the cause of AVM. If the agent is only present for a short window of time, the presence of neurologically abnormal or debilitated coots may not indicate concurrent presence of the causative agent. It would be informative to conduct feeding studies that use material collected concurrent with the first onset of AVM in wild or sentinel birds.

The authors thank J. Itenson, G. Kalema, C. Whittier, E. Chittick, A. Acton, S. Willens, A. Moresco, and North Carolina State University faculty, staff, residents, and veterinary students for technical assistance and logistic support. Funding was provided, in part, by the Environmental Medicine Consortium, NCSU, the North Carolina Zoological Society, and the US Fish and Wildlife Service Division of Environmental Contaminants (Study ID 200040002.1).

LITERATURE CITED

BENNETT, R. A. 1994. Neurology. *In* Avian medicine: Principles and application, B. W. Ritchie, G. J. Harrison, and L. R. Harrison (eds.). Wingers Publishing Co., Lake Worth, Florida, pp. 723–747.

CARLTON, W. W., AND G. KREUTZBERG. 1966. Isonicotinic acid hydrazide-induced spongy degeneration of the white matter in the brains of pekin ducks. American Journal of Pathology 48: 91–105.

FLEMING, W. J., E. F. HILL, J. J. MOMOT, AND V. F. PANG. 1991. Toxicity of trimethyltin and triethyltin to mallard ducklings. Environmental Toxicology and Chemistry 10: 255–260.

HARRISON, G. J., AND B. W. RITCHIE. 1994. Making distinctions in the physical examination. *In* Avian medicine: Principles and application. B. W. Ritchie, G. J. Harrison, and L. R. Harrison (eds.). Wingers Publishing Co., Lake Worth, Florida, pp. 144–175.

LAMPERT, P. W., AND S. S. SCHOCHET. 1968. Electron microscopic observations on experimental spongy degeneration of the cerebellar white matter. Journal of Neuropathology and Experimental Neurology 27: 210–220.

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

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

ROWE, B. L., AND H. H. PRINCE. 1983. Behavioral response of mallards to contaminated drinking

water. Bulletin of Environmental Contamination and Toxicology 30: 505–510.

TAYLOR, P. B. 1998. Rails: A guide to the rails, crakes, gallinules and coots of the world. Yale University Press, New Haven, Connecticut, 600 pp.

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.

Received for publication 17 December 2001.