



National Wildlife Health Center

...advancing wildlife and ecosystem health for a better tomorrow

Workshop on Avian Vacuolar Myelinopathy

United States Geological Survey, Biological Resources Discipline
National Wildlife Health Center

October 26-27, 2000

Introduction and Background:

On October 26 and 27, 2000, the USGS/BRD National Wildlife Health Center (NWHC) hosted a half day technical workshop to discuss potential mechanisms and causes of avian vacuolar myelinopathy (AVM), an emerging neurologic disease of wild birds in Southeastern U.S. AVM was first recognized in bald eagles at DeGray Lake, Arkansas in 1994, and 2 years later, the disease was confirmed in a number of coots on this and another lake in Arkansas (Thomas, et al. 1998. Epizootic vacuolar myelinopathy of the central nervous system of bald eagles and American coots. *Veterinary Pathology*. 35:479-487). Since then, AVM has been confirmed or suspected in coots on 11 lakes in 5 states (Arkansas, North Carolina, South Carolina, Georgia, and Texas) and also in several species of waterfowl (mallards, ring-necked ducks, bufflehead ducks and Canada geese). The disease is characterized by a specific lesion in the myelin of the brain and spinal cord of affected birds, but despite extensive diagnostic and field investigations, the causative agent of AVM is still unknown.

The AVM workshop, sponsored by the USGS Biological Resources Discipline, was convened by NWHC to enlist the help of scientists with specialized expertise in myelin biochemistry, neurotoxicology and neuropathology to review the underlying biochemical mechanisms of myelin function and structure and to further our efforts to identify the cause and sources of AVM ([Attachment 1](#)). Others in attendance included representatives from the U.S. Fish and Wildlife Service, Southeastern Cooperative Wildlife Disease Study, the Ross Foundation, and numerous scientists from NWHC. The workshop began with brief overviews by participants of previous field investigations of AVM outbreaks, diagnostic testing, and research studies to date. Several of the pathologists in attendance also reviewed slides and other case material. The discussion was then focused on a series of core questions that were posed specifically to the panel ([Attachment 2](#)) and their recommendations for further research and diagnostic activities.

The invited panel was very encouraging and positive about the work to date on this disease, and had many good suggestions and recommendations. To briefly summarize the discussion, the panel concurred that:

- The NWHC pathologists' morphological characterization of the brain lesion was correctly described as a vacuolar myelinopathy, compatible with intra myelinic edema.
- The single most important epizootiologic finding to date was recreation of the disease in sentinel birds within 5 days of placement in an affected lake.
- Mallards are a suitable sentinel animal for AVM, as confirmed by sentinel studies.
- The lesion and epizootiology suggest that AVM is caused by a "chemical substance" of unknown origin; microbial or other natural toxins are chief suspects.
- Detection of the lesion in subjects with no apparent clinical signs is not an uncommon finding in research animals with experimentally-induced myelin disorders of this nature.

- Myelin structure and function is similar enough between vertebrates that we might expect to see the disease in other animals if exposed; a similar lesion has been observed in animals and humans exposed to certain chemicals (hexachlorophene and triethyl tin).
- The potential risk to humans should not be ignored.
- Sentinel studies are critical at this point and the most logical way to proceed to determine the source and causative agent of AVM. Woodlake, a small, easily navigated lake where AVM outbreaks have occurred annually in coots since 1996 is the most appropriate location for study.
- Findings to date should be communicated as soon as possible in a variety of outlets (professional meetings, journals etc.).
- Identifying the causative agent will require multi-year support

Panel Recommendations:

A. Continue and expand current sentinel studies and bioassays for AVM:

1. Determine the source of exposure by partitioning exposure to food, sediment, water, and air in sentinel mallards or coots.
2. Expand investigations into the species affected by examining fish and mammals in affected lakes for evidence of brain lesions.
3. Identify the food habits of AVM-affected birds by collecting and identifying their gut contents.
4. Expand clinical, clinical pathology and pathology characterization of AVM-affected birds by collecting additional organs from sentinel birds for pathologic examination; use intravascular fixative perfusion techniques in a limited number of sentinels to optimally prepare tissue for pathologic examination; measure packed cell volumes and body temperature of affected birds; collect cerebrospinal fluid.
5. Document site characteristics during sentinel studies by collecting samples of surface water for algae examination and by measuring local water characteristics.

B. Develop a laboratory animal model for use as a bioassay in epidemiologic investigations.

1. Determine whether mice or ducklings may serve as a laboratory bioassay for AVM. Feed livers and gut contents from AVM-affected birds to mice and ducklings and also consider cats and chickens, as neurologic changes may be more easily detected clinically in these species.
2. Use a positive control in all feeding studies (i.e. hexachlorophene or triethyl tin).
3. Note that the tadpole has been employed previously to assess in vivo the status of central myelin (optic nerve) and peripheral nerve myelin in normal and myelinotoxic states. Explore whether the tadpole could serve as a simple biomonitor of water safety in AVM-affected areas.

C. Conduct site characterization of affected lakes with paired controls (case control study)

1. Identify characteristics in common among sites where AVM has occurred by retrospective analysis of:
 - water temperature fluctuations*
 - land use, population density, vegetation*
 - limnological parameters*
 - water quality, water softness*
 - physical characteristics*
 - chemical use inventories*
 - meteorologic data*

2. Test risk hypotheses developed from retrospective analyses and fill in data gaps by site assessment and characterization during ongoing AVM outbreaks. Collect additional data as needed; measure water characteristics during AVM outbreaks and conduct microbial surveys.

D. Communicate findings to date as soon as possible.

1. Increase communication about ongoing AVM investigations with other federal agencies, including CDC, USDA, EPA.
2. Publish preliminary sentinel findings immediately as a short note to alert biologists, managers, and others to the site specificity of AVM and the short time of onset.
3. Consider writing a short communication in an international outlet such as Lancet or Science, describing the disease, calling for similar reports, and addressing the potential human health risk; panel may consider submitting this as a group.
4. Consider describing AVM in a special symposium at the Society of Toxicology annual meeting.

E. Formally invite the workshop participants to serve as a technical advisory panel on AVM; consider including expertise in epidemiology, limnology, algal toxins, statistics and public health (risk assessment).

Actions to be taken as a result of the workshop:

NWHC will take immediate action on several of the suggestions and recommendations that resulted from the workshop.

A. A sentinel study has just begun on Woodlake, NC and will be expanded. Fish will be sampled biweekly and examined for brain lesions, and attempts will also be made to collect mammals opportunistically. Core body temperature and PCV's will be measured in affected birds, in addition to other blood parameters currently being measured, and a sample of all tissues will be collected and fixed upon necropsy, including GI contents for later identification of food items. A sample of affected coots will be anesthetized and then perfused with fixative for light and electron microscopy; cerebrospinal fluid will be collected from these birds prior to perfusion. Water samples will be collected periodically to survey algae populations and various water characteristics will be measured weekly.

B. Feeding studies in mallards removed from the site will also begin soon as previously planned to partition possible sources of exposure. Food items and water will be collected from Woodlake during AVM outbreaks, transported to North Carolina State University, and administered to mallards held in pens. The mallards will be observed for signs of disease; sick birds will be euthanized and brains will be collected for histologic examination. After 7-10 days of exposure, all birds, including apparently healthy birds, will be euthanized and their brains collected for histologic examination.

C. Experiments are being planned to feed livers and GI contents from affected birds to mice and ducklings (in collaboration with FWS and SCWDS) to identify an appropriate laboratory animal model, and these studies will begin as soon as possible. Triethyl tin will be used as a positive control as it has been shown to cause a similar lesion in mallards previously. Tadpoles may also be considered as a model.

D. Previous proposals have been submitted for site characterization studies (case control epidemiology studies) and will be submitted to various outlets again, but to date no funding has been obtained.

E. A short communication on the preliminary sentinel findings will be written and submitted for publication immediately. Other outlets will be considered, including letters to Lancet or Science and special symposia. Efforts will be made to alert other federal agencies about AVM and our investigations, particularly when outbreaks occur.

Acknowledgment: We are grateful to the U.S. Geological Survey, Biological Resources Discipline, for funding this workshop on AVM.

Attachments:

AVM Workshop - Invited Panel

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<p>Dr. Kinuko Suzuki Pathology and Lab Medicine 401 Brinkhous-Bullitt Bldg University of North Carolina Chapel Hill, NC 27599</p>	<p>Dr. Karl Jensen Environmental Protection Agency Natl. Health and Environmental Effects Research Triangle Park, NC 27709</p>
<p>Dr. Raymond Baggs Dept. Of Laboratory Animal Medicine University of Rochester Medical Center Rochester, NY 14642</p>	<p>Dr. Peter Spencer Center for Research on Occupation and Environmental Toxicology Oregon Health Science University 3181 SW Sam Jackson Park Rd. L606 Portland OR 92701</p>
<p>Dr. Brian Popko Neuroscience Center, CB #7250 University of North Carolina Chapel Hill, NC 27599</p>	<p>Dr. Ian D. Duncan Medical Sciences School of Veterinary Medicine 2015 Linden Dr. W Madison, WI 53706</p>
<p>Dr. Diane Henshel School of Public and Environmental Affairs Environmental Neurotoxicology Spea Building 1315 E. 10th St. Bloomington, IN 47405</p>	

Other Workshop Participants:

<p>Tom Augspurger US Fish and Wildlife Service Ecological Services Raleigh, North Carolina</p>	<p>Dr. Joe Nix Ross Foundation P.O. Box 335 Arkadelphia, AR 71923</p>
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