The Northeast Wildlife Disease Cooperative

Offering wildlife health and disease services in the Northeast U.S.

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NWDC NOTES

QUARTERLY NEWSLETTER FROM THE NORTHEAST WILDLIFE DISEASE COOPERATIVE

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NWDC Training Workshops

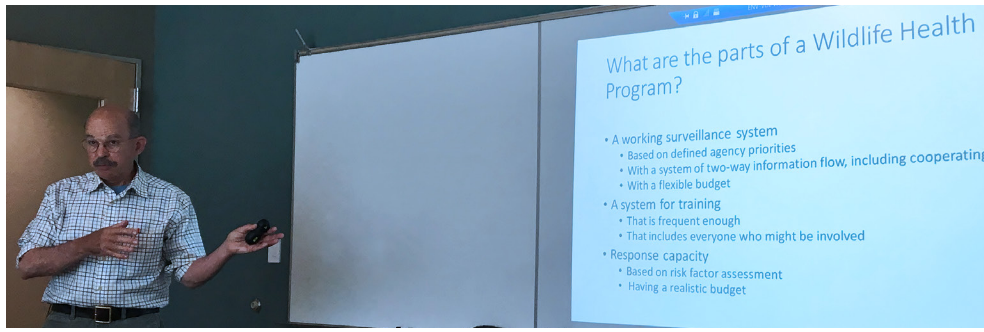

Figure 1. Walt Cottrell presenting on the components of a Wildlife Health Program.

As most of you already know, the training workshops this year represented a major departure from those given previously. As we began thinking about our goals for these workshops, it became clear that it was time for a change. Several trends guided our thinking, among them, that lab submissions remained low and that we were still getting questions from members about whether a submission was warranted. The response to the Problem-Based Learning Exercises from last year's workshops also indicated that the "on the ground" decision making process in many states lacked structure. The unavoidable analogy was that we had provided our members with the viscera (facts) of a Wildlife Health Program, but that the muscles (the investigations and submissions) could not function because the skeleton (structured criteria) was not in place to give it strength or provide a reference point for daily decisions.

In light of this significant gap, we asked administrators to bring together those in their agency with the greatest species knowledge, administrative assistants who answer the phones, and representatives from some important collaborating agencies. To continue the anatomic analogy, we asked the eyes and ears of the agency to participate in developing the needed structure. The resultant workshops were long days, but the process provided a beginning point for a truly functional Wildlife Health Program in each state. Based on the input and discussion at the workshops, we generated three Surveillance Plans (one each for mammals, birds, and herps) for member agencies. The Plans delineate a systematic way to determine which wildlife mortality events warrant action, and whether the action is simply documentation or submitting samples to a diagnostic lab. As such, the Plans include a table of priority species and mortality circumstances (e.g numbers and species of animals affected, case definitions) as defined by each agency. This table is followed by an Action Plan that includes a list of specific questions to ask regarding the history of a mortality event, steps to take before and after a diagnosis is received, an internal agency notification tree, and roles of potential collaborating organizations. The Surveillance Plans are living documents that will surely need review and revisions over time, but they are intended to form a solid "skeleton" for a wildlife health program.

Investigating the Impacts of West Nile Virus on Ruffed Grouse in Pennsylvania

Justin Brown, PhD, DVM, Wildlife Veterinarian and Lisa Williams, Grouse Biologist

Pennsylvania Game Commission

The ruffed grouse (*Bonasa umbellus*) is a forest-dwelling, non-migratory, upland game bird found throughout Canada and much of the northern US. Early successional forest habitats are critical for ruffed grouse by providing cover for protection and breeding as well as a diversity of food resources. Unfortunately, ruffed grouse populations are declining throughout much of the Appalachian and mid-Atlantic region. The primary driver of the decline is thought to be widespread maturation of forests and loss of early successional habitat.

Pennsylvania ruffed grouse populations are currently at their lowest point in 50 years of monitoring. While the mature forests blanketing Penns Woods are likely a primary contributor to this outcome, a closer look at the population trends over the last 15 years suggests an additional factor could be involved. In northern portions of their range, grouse populations follow a classic "boom-and-bust" cycle with dramatic peaks and valleys occurring over a 5 to 10 year period. While the magnitude of population fluctuations in the central and southern Appalachians is not as great, cyclical increases and decreases are still observed. During 2001-2004, the grouse population in Pennsylvania experienced a precipitous decline (i.e. a bust), which in itself is not atypical. However, no boom has followed since that time and statewide population estimates have remained suppressed below the long-term 50 year average. Similar declines in ruffed grouse populations were observed in many of the surrounding Appalachian states during 2001-2004. Several factors were initially considered to explain the observed population trends, including predation, weather, and habitat. None of these factors seemed to account for what was being observed in Pennsylvania grouse, as they would presumably occur gradually over time, not result in the long-term suppression of the population, and/or have localized (rather than regional) impacts. Temporally, the declines were associated with the expansion of West Nile virus (WNV) throughout Pennsylvania during 2000-2002, which initially sparked our interest. Upon closer inspection, when annual Pennsylvania grouse population estimates were compared to statewide WNV data from mosquitos or dead birds, there was a clear pattern of abrupt declines in grouse populations following years of increased WNV activity. While significant ruffed grouse mortality due to WNV has not been reported in any state, it certainly could not be ruled out as a factor in the observed population declines because of inherent problems with detecting mortality in a medium-sized, highly-camouflaged forest bird. In order to better define the impacts of WNV on ruffed grouse, the Pennsylvania Game Commission initiated a collaborative project with Colorado State University, University of Guelph, Snyder's Grouse Park, Ruffed Grouse Society, and Woodcock Limited. The primary research objectives were: 1) to determine the susceptibility of ruffed grouse to WNV, and 2) to measure exposure to WNV in ruffed grouse throughout Pennsylvania.

For Objective 1, 18 juvenile, wild-collected grouse raised in mosquito-proof enclosures were involved in a 14 day experimental infection study, including 10 naïve birds challenged with WNV, 5 vaccinated birds challenged with WNV, and 3 sham-inoculated negative controls. Out of the 10 naïve grouse exposed to WNV, 4 became severely-ill and had to be euthanized on day 7 or 8 post-inoculation. An additional 5 of the remaining 6 naïve WNV-exposed grouse survived to the end of the trial but had moderate to severe lesions in the heart and/or brain as a result of WNV infection. While it is always challenging to assess the clinical impacts of lesions observed in a laboratory setting for grouse in the wild, the severity of the inflammatory and necrotizing lesions would presumably have some effect on survival. None of the vaccinated WNV-exposed grouse or negative controls exhibited clinical disease or had significant lesions associated with WNV infection. With 90% of the naïve birds experiencing significant disease and/or lesions in critical organs, the experimental data indicate grouse are highly susceptible to WNV infection.

Figure 2. A blood soaked Nobuto filter paper strip and blood sample.

For Objective 2, antibodies to WNV will be measured in hunter-harvested grouse throughout Pennsylvania. Because of inherent challenges in obtaining serum samples from hunter-harvested grouse, Nobuto paper strips were utilized for this WNV serosurvey. Nobuto paper strips are an inexpensive sampling approach that can be used to circumvent problems relating to low volumes of blood, maintaining cold chain of samples, and the need for processing samples soon after collection. The paper strips are simply soaked in fresh blood in the field, air dried, and the antibodies eluted and tested once back in the laboratory (Figure 1). Nobuto paper strips have been successfully used for WNV detection in wild birds previously and were further validated for ruffed grouse on a limited number of samples collected from experimentally-infected birds involved in Objective 1. During the first year of sampling, 202 Nobuto paper strips were collected from ruffed grouse harvested in 32 Pennsylvania counties. Preliminary data indicate 13.8% of these grouse samples had antibodies to WNV. We will collect samples again this upcoming hunting season in order to increase our sample size, particularly in the southern half of the state.

Current efforts are underway to collaborate with ecologists and incorporate the WNV data we are collecting into grouse population models. Our ultimate goals are to define interactions and impacts of WNV and habitat on grouse population dynamics, and ultimately identify management options that may support or recover grouse populations in our state and region.

Preliminary Findings in Four Dead Canada Lynx (*Lynx canadensis*) from Maine

David Needle, Brian Stevens, NHVDL

Histopathology: All four animals had indirect evidence of lungworm infection characterized by significant inflammation. Three of these animals had sections of adult and larval lungworms on histopathology. These lesions consisted of eosinophils, the white blood cell most associated with parasitic infection, infiltrating the airways in the lungs. The inflammatory cells were accompanied by increased production of mucous, resulting in partial blockage of these airways. Overall, these lesions were consistent with the type of inflammatory reaction (type I hypersensitivity) commonly attributed to parasitism. The severity of these lesions varied from mild to severe. The cause of death in most cases was related to trauma, and it is unclear at this point what effect this parasitic pneumonia had on the animals.

There were also encysted, inactive aggregates of *Sarcocystis*-like parasites in skeletal and cardiac muscle in all animals. The genus *Sarcocystis* is a group of protozoan parasites that commonly colonize skeletal muscle wild animals. The majority of the time there is no disease associated with these parasites, as is likely the case in these lynx. In non-target hosts aberrant migration of the parasite can lead to significant lesions and illness.

In addition to the lung worms and encysted protozoa, one animal had multifocal inflammation in multiple skeletal muscle bellies, and another had a single similar focus in the skeletal muscle of the tongue. These findings consisted of aggregates of lymphocytes within the muscle. This inflammation was not associated with an obvious pathogen. The overall importance of this finding to the animals' health is not clear, but it is of interest since having multiple muscles affected in one animal might indicate a degree of systemic involvement.

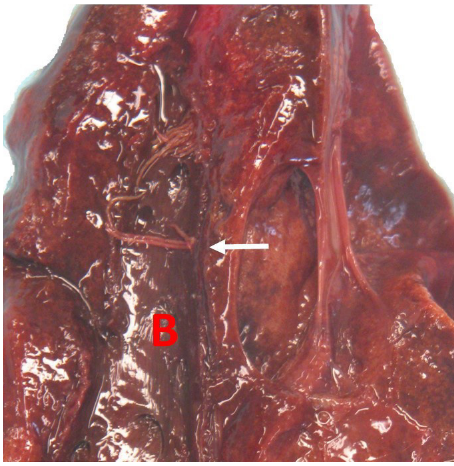

Figure 3. A section of lung, with nematodes (indicated by white arrow) within a bronchus (B).

Parasitology: Lungworm larvae were collected via a Baerman technique and were consistent with *Troglostrongylus* sp. Adult ascarids and cestodes were identified from specimens collected during necropsy, and their eggs were identified during fecal flotation. The cestode eggs were most consistent with *Taenia* sp. A single animal had a single trichuroid (whipworm) egg that had characteristics of *Trichuris* sp., but a size more consistent with *Capillaria* sp.

Future directions: The remainder of the animals in the study set (N = 36) will be necropsied. Selected tissues will be examined for histopathological lesions, and a parasitology workup will be undertaken. For the four animals already examined and those that will be examined, samples of lung, liver, kidney, spleen, and lymph node were frozen for potential future studies.

Overall, the quality of the tissues recovered from the animals examined this far have been fair, with the effects of freezing and thawing fairly noticeable. The most effective way to get the best samples, and thus the best data from each animal, is for a field necropsy to be performed as soon as the animal is found. Representative sections of the tissues can be placed in formalin, and portions of pertinent organs can be stored on ice or frozen immediately as well. As an added bonus, the cost for examination of tissues obtained during a field necropsy is far less than the cost of a full necropsy, and the transport of a formalin container is far easier and less expensive than a whole carcass. The NWDC and NHVDL is exploring this training with Maine Department of Inland Fisheries and Wildlife.