SUSCEPTIBILITY OF RUFFED GROUSE \textit{(Bonasa umbellus)} TO WEST NILE VIRUS

Nicole M. Nemeth\textsuperscript{1}, Angela M. Bosco-Lauth\textsuperscript{2}, Lisa M. Williams\textsuperscript{3}, Richard A. Bowen\textsuperscript{2}, Justin D. Brown\textsuperscript{3}
Department of Pathobiology, University of Guelph, Canada\textsuperscript{1}, Department of Biomedical Sciences, Colorado State University\textsuperscript{2}, Pennsylvania Game Commission, Bureau of Wildlife Management,\textsuperscript{3} United States.

**Background**
- The ruffed grouse is an important gamebird and the state bird of Pennsylvania (PA).
- West Nile virus (WNV) arrived in the northeastern U.S. in 1999 and spread throughout PA by 2002.
- Grouse populations in PA declined precipitously from 2002-2005. Robust recovery has not occurred (Fig. 1).
- To assess potential impact of WNV as a contributing factor in grouse declines, a challenge study of juvenile wild-collected grouse was conducted.

**Methods**
- Grouse eggs were collected in spring 2015 from 7 nests representing a longitudinal gradient in PA.
- Chicks were hatched and raised in mosquito-proof enclosures.
- 18 juveniles (7-8 weeks of age) were included in a 14 day experimental infection study.
- Test groups included:
  - 10 naïve birds inoculated with WNV
  - 5 vaccinated birds challenged with WNV
  - 3 sham-inoculated negative contact controls
- All WNV-inoculated birds, including vaccinated and naïve, were injected subcutaneously with 0.1 ml (titer: 1.3 x 10\textsuperscript{4} PFU/ml) of a geographically- and temporally-relevant strain of WNV (isolated from \textit{Culex pipiens restuans}, Suffolk County, NY, 2014).
- Grouse were monitored twice daily for clinical signs.
- **Virus shedding:** Blood was collected from each bird on 0-7 days post-inoculation (DPI). Oropharyngeal and cloacal swabs were collected on 2-5 DPI. Virus isolation and titration was performed on all swab and blood samples using Vero cell plaque assay.
- **Antibodies:** Blood was collected from all birds prior to WNV inoculation and when they were euthanized. Serum was tested for antibodies to WNV using a plaque-reduction neutralization test.
- Birds showing clinical signs were euthanized immediately; all others were euthanized on 14 DPI.
- Necropsies were performed on all birds. Gross lesions were identified and representative samples were collected from major organs for histopathologic and immunohistochemical examination.

**Results**
- Forty percent (4/10) of naïve inoculated birds were euthanized at 7-8 DPI due to severe clinical disease (e.g., weight loss, dehydration, hind limb paresis); lesions in these birds included severe non-suppurative myocarditis, myocardial degeneration and minimal encephalitis (Table 1, Fig. 2).
- In grouse that survived to 14 DPI, encephalitis was more severe and half also had severe myocarditis (Table 1), suggesting that encephalitis is more likely a chronic manifestation of WNV in grouse. These lesions occurred without overt clinical signs of disease.
- No in-contact sham-inoculated controls or vaccinated birds had clinical signs of disease or significant lesions.
- Peak viremia titers were moderate (mean peak: 10\textsuperscript{6.9} pfu/ml serum). Viremia usually lasted 4-6 days, and up to 8 days in birds euthanized due to illness.
- All inoculated grouse surviving to 14 DPI developed antibodies.

**Conclusions**
- 40% of the naïve ruffed grouse inoculated with WNV died acutely (i.e., 1 week following infection). WNV replicated and caused lesions in multiple tissues but damage was most severe in the heart.
- 5/6 WNV-inoculated naïve grouse surviving to the end of the trial had severe lesions in the brain and heart. Long-term survival in these birds would likely have been compromised due to the severity of lesions.
- A single vaccine dose protected grouse from WNV-associated lesions.
- Nobuto filter strips were validated as an effective way to sample ruffed grouse for WNV antibodies.
- Collectively, 90% of naïve birds inoculated with WNV experienced significant disease/lesions in critical organs, indicating grouse are highly susceptible to WNV infection.

**Acknowledgments**
We thank all who helped locate grouse nests and Dan Snyder (Grouse Park Waterfowl, Idaho) for his generosity and expertise in hatching, raising and transporting ruffed grouse.