SUSCEPTIBILITY OF RUFFED GROUSE (*BONASA UMBELLUS*) TO WEST NILE VIRUS

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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 10^4 PFU/ml) of a geographically- and temporally-relevant strain of WNV (isolated from *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.

Figure 1. Grouse population indices pre- and post-WNV introduction in Pennsylvania.

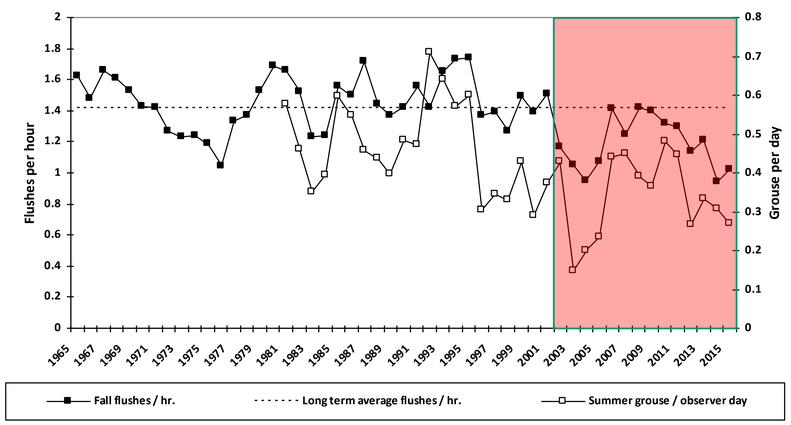
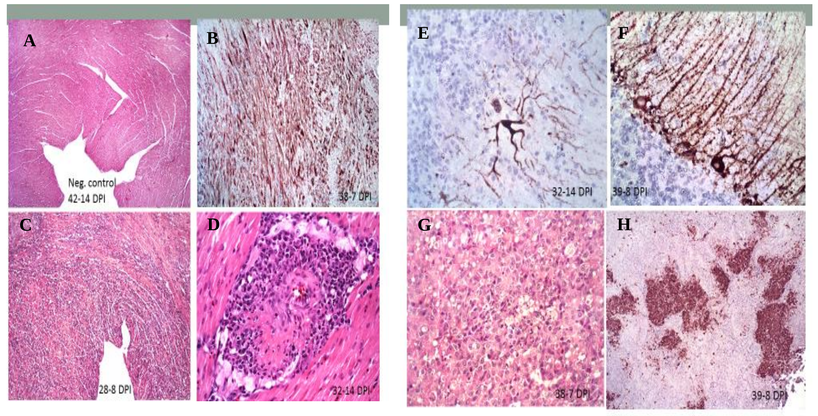


Table 1. Microscopic lesions and immunohistochemical staining in tissues from ruffed grouse inoculated with WNV. The proportion of each experimental group affected and severity of lesions are depicted.

	Clinically affected, non-vaccinated (n=4)			Subclinical, non-vaccinated (n=6)			Vaccinated (n=5)		
Tissue	INFL	NECR	IHC[a]	INFL	NECR	IHC	INFL	NECR	IHC
Heart	4/4; +++	4/4; +++	3/4; + to +++	6/6; ++ to +++	6/6; + to +++	1/6; +[b]	2/5; +	0/5	0/5
Cerebrum	2/4; +	0/4	1/4; +	3/6; +	0/6	0/6	1/5; +	0/5	0/5
Cerebellum	2/4; +	0/4	2/4; + to ++	6/6; + to +++	0/6	1/6	3/5; +	0/5	0/5
Pancreas	2/4; +	3/4; + to +++	4/4; +	4/6; +	0/6	0/6	1/5; +	0/5	0/5
Adrenal gland	2/4; ++	3/4; ++ to +++	4/4; + to +++	1/6; ++	2/6; + to ++	3/6; +	1/5; +	0/5	0/5
Duodenum	3/4; +	3/4; +	3/4; +	0/6	0/6	0/6	0/5	0/5	0/5
Kidney	3/4; +	0/4	3/4; +	5/6; + to ++	3/6; +	0/6	0/5	0/5	0/5
Liver	3/4; +	1/4; ++	NT	5/6; +	0/6	NT	3/5; +	1/5; +	NT

[a]Immunostaining assessment: 0 = no staining; + = ≤5% of tissue section(s) with positive staining; ++ = 6-25% of tissue section(s) with positive staining; and +++ = >25% with positive staining. NT=not tested; INFL=inflammation; NECR=necrosis; IHC=immunohistochemical staining.

Figure 2. WNV-associated lesions (HE stain and IHC) in ruffed grouse heart (A-Control; B-IHC+; C-Diffuse inflammation; D-Perivascular inflammation); cerebellum (E,F-IHC+); pancreas (G); adrenal gland (H-IHC+)



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: $10^{6.9}$ 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

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