Surveillance for Lymphoproliferative Disease Virus in Hunter-Killed Eastern Wild Turkeys (*Meleagris gallopavo*)

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Viral-induced Tumors in Gallinaceous Birds

- Herpesvirus (Marek’s Disease)

- Retroviruses
  - Avian leukemia/sarcoma viruses
  - Reticuloendotheliosis virus (REV)
  - Lymphoproliferative disease virus (LPDV)

Historically, only REV was reported from wild upland game birds.
History of LPDV

• First described in domestic turkeys in the United Kingdom in 1972

• Sporadic outbreaks reported in other European countries and Israel

• Apparently a restricted host range
  – Natural infection only reported in domestic turkeys
  – Turkeys and chickens have been infected experimentally

• Considered poultry pathogen of minor importance globally
LPDV in the U.S.

- First recognized in the United States in 2009
  - 3 adult Eastern wild turkeys with lymphoid tumors
    - 2 from West Virginia, 1 from Arkansas
  - Negative for REV, pox
  - Positive for LPDV
Histopathology

Liver (cc12-09)

Skin (cc152-11)

Tumors composed of neoplastic lymphoid cells
Eastern Wild Turkey Clinical Cases ‘09-’12

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26 LPDV positive clinical cases from 14 states
Testing for LPDV

- Method of *in vitro* culture not currently known

- Diagnostics rely on histopathology and PCR
  - Lesions not specific to LPDV
  - Lesions may not always be present

- PCR testing
  - Primers that target a 457 bp portion of the gag gene
Objectives

• Determine the prevalence and distribution of LPDV in apparently asymptomatic hunter-killed Eastern wild turkeys.
  – Analyze differences among different age and sex classes.

• Determine best tissue for LPDV detection in asymptomatic turkeys.
  – Spleen, bone marrow, liver
Sample Collection and Testing

- Liver, spleen, and bone marrow collected from hunter-killed wild turkeys
  - Tissues selected based on
    - Literature (LPDV and REV in domestic turkeys)
    - Experience (LPDV in wild turkey clinical cases)
    - Availability and compliance (Infrastructure and trained personnel)
- To date, 285 samples have been collected from 5 states
  - Spring 2011: South Carolina (n=68)
  - Spring 2012: West Virginia (n=42)
  - Fall 2012: New York (n=107)
    - Missouri (n=33)
    - New Jersey (n=35)
- Testing by PCR as previously described
### Results

<table>
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<th>State</th>
<th>Tissue Type</th>
<th>Number Sampled</th>
<th># Positive (%)</th>
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<td>Missouri</td>
<td>Liver</td>
<td>33</td>
<td>8 (24%)&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>New Jersey</td>
<td>Bone Marrow</td>
<td>35</td>
<td>13 (37%)&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>New York</td>
<td>Bone Marrow</td>
<td>107</td>
<td>44 (41%)&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<td>South Carolina</td>
<td>Liver</td>
<td>68</td>
<td>33 (49%)&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<tr>
<td>West Virginia</td>
<td>Liver</td>
<td>42</td>
<td>68 (50%)&lt;sup&gt;b,c&lt;/sup&gt;</td>
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</table>

*Numbers with the same letter are not significantly different from each other
**No significant difference between tissue type tested
LPDV Distribution

South Carolina

West Virginia

New York

Missouri

New Jersey

LPDV Results
- Not Tested
- Positive
- Negative
LPDV Detection Among Different Age/Sex Groups in New York

Adult Males vs. Adult females (p=0.79)
Juv. Males vs. Juv. Females (p=0.53)
Adults vs. Juveniles (p=0.23)
Males vs. Females (p=0.84)
LPDV Detection Among Different Age/Sex Groups from NY, MO, and NJ

**Diagram:**

- **Adult Male:** n=51
- **Adult Female:** n=46
- **Juv. Male:** n=26
- **Juv. Female:** n=46

**Statistical Tests:**

- Adult Male vs. Adult Female (p=0.84)
- Juv. Male vs. Juv. Female (p=0.12)
- Adults vs. Juveniles (p=0.2)
- Males vs. Females (p=0.34)
Conclusion

- High prevalence of LPDV proviral DNA among Eastern Wild Turkeys
  - Clinical disease is uncommon
  - We currently do not know whether these positives represent whole virus or segments of viral DNA

- Widespread across the eastern United States

- Appears that the virus has been in the U.S. for a long period of time
  - Misdiagnosed and not looked for until now
Future directions

• Increase sample size
  – Expecting samples from at least 11 more states
  – More samples from 5 states presented today
• Collection of multiple samples from individual turkeys to evaluate best tissue for diagnostics
• Targeted surveillance of additional wild turkey subspecies and wild galliform species
LPDV Future Studies

- Genetically characterize the North American wild turkey LPDV
  - Allison (Cornell Univ.)
- Characterize epidemiology in wild turkeys
  - Brown (UGA)
- Determine how to culture virus
  - Zavala (PDRC), Fadley (USDA-ARS)
- Characterize pathobiology
  - Zavala (PDRC)
Acknowledgements

• Numerous wildlife biologists and veterinarians
  – James Crum (WVDNR), Elizabeth Bunting (Cornell Univ.),
    Kevin Hynes (NYS DEC), Tony McBride (NJDFW), Jason
    Isabelle (MDC), and SCDNR

• Funding provided through the continued support of
  SCWDS by member states and federal agencies.