Avian Disease & Oncology Lab (ADOL) Research Update

John Dunn, Hans Cheng, Mohammad Heidari, Huanmin Zhang

USDA-ARS-USPNRC
Research Programs at ADOL

1) **Genomics (Cheng, Zhang, Vacant SY)**
   Title: Employing genomics, epigenetics, and immunogenetics to control diseases induced by avian tumor viruses

2) **Tumor Virus (Dunn, Heidari, Vacant SY)**
   Title: Genetic and biological determinants of avian tumor virus pathogenicity, transmission, and evolution
Improved Genetic Map for Chicken Genome Assembly

• Despite chicken being the first agricultural animal with a genome assembly (2004) and several updates, it is still incomplete, e.g., 8 of the 38 autosomes lack any sequence information.

• To aid in the most recent assembly (galgal5), genetic markers from unplaced sequence contigs were added to the East Lansing genetic map.
Chicken assembly galgal5.0 status

- Assigned sequence to 33 of 38 autosomes
- 29 new linkage groups defined
- Contiguity is 10-fold better
- Increased representation by ~180Mb
- Yet, 138 Mb of sequence unassigned
Marek’s disease virus infections influence chicken gut colonization

Days 14, 21: *Blautia* spp.
- Succinate
- Lactate

Day 28: *Streptococcus* spp.
- Acetate

Days 35, 42: *Faecalibacterium* spp.
- Butyrate

Promotes colon health in humans against cancers

Healthy Chicken

Under MDV infection
Identify Driver Genes for Marek’s Disease Tumors

1,000 pfu MDV (JM/102W) 

Line 6 (resistant) x 7 (susceptible) 

200 Birds 

4-8 Weeks 

Tumor Samples 

Transformation and Tumorigenesis
IKZF1

IKZF1: IKAROS Family Zinc Finger 1

• Associated with T-cell development
  – Important role in CD4 vs. CD8 lineage commitment

• Tumor Suppressor Gene
  – Acute Lymphoblastic Leukemia (ALL)

• Significantly mutated across samples (19%)

• Deleterious mutations (dominant negative)
Recent MDV isolates

Farms with Any Poultry Inventory on December 31, 2012

United States Total
233,770

1 Dot = 20 Farms

2012 Census of Agriculture

U.S. Department of Agriculture, National Agricultural Statistics Service
New strains not more pathogenic

Virulence rank (red = new cases)
32 genomic regions used for targeted sequencing

Projected gene of targeted regions

<table>
<thead>
<tr>
<th>Protein</th>
<th>U_L Genes</th>
<th>U_S Genes</th>
<th>Protein</th>
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<tbody>
<tr>
<td>R-LORF1</td>
<td>UL15</td>
<td>UL41</td>
<td>R-LORF7</td>
</tr>
<tr>
<td>R-LORF5</td>
<td>UL15</td>
<td>UL43</td>
<td>R-LORF7</td>
</tr>
<tr>
<td>R-LORF7</td>
<td>UL22</td>
<td>UL47</td>
<td>R-LORF5</td>
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<td>UL36</td>
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<td>R-LORF8</td>
<td>UL36</td>
<td>LORF11</td>
<td>ICP4</td>
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<td>UL6</td>
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<td>SORF1</td>
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<td>?</td>
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<td>UL9</td>
<td>LORF7</td>
<td>R-LORF8</td>
<td>ICP4</td>
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</tbody>
</table>

Amplicons 200-400bp, Illumina MiSeq (2x250bp PE reads)
Diversity of sequenced isolates
Location, virulence & time

15 vMDV (1962-2014)
24 vvMDV (1977-2011)
25 vv+MDV (1990-2013)
phyML
TreeView
### Preliminary association results

<table>
<thead>
<tr>
<th>Low Vr Base</th>
<th>% of strains with Low Vr Base</th>
<th>High Vr Base</th>
<th>Gene</th>
<th>AA</th>
<th>Protein Position</th>
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<tbody>
<tr>
<td>A</td>
<td>94 63 40</td>
<td>C</td>
<td>UL22</td>
<td>M-&gt;R</td>
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<tr>
<td>G</td>
<td>94 42 16</td>
<td>A</td>
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<td>T</td>
<td>94 29 0</td>
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<tr>
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<td>UL37</td>
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<tr>
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<td>72 21 8</td>
<td>T</td>
<td>ICP4</td>
<td>P-&gt;S</td>
<td>180</td>
</tr>
</tbody>
</table>
## Effect of probiotics on MD vaccination

<table>
<thead>
<tr>
<th>Company A</th>
<th>Live lactic acid bacteria (LAB) probiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company B</td>
<td>Yeast fermentation product (YFP) <em>(Saccharomyces cerevisiae)</em></td>
</tr>
</tbody>
</table>
Trials 1 & 2: %MD reduced

%MD (challenged with 583A)

- HVT (in ovo): p=0.1850
- HVT + LAB (in ovo): p=0.0785
- HVT (in ovo) + YFP (Feed)

Marek’s disease (%MD) reduced in Trials 1 & 2.
Trial 3: Less effect with more virulent strains

%MD (challenged with Md5 or 612)

% Marek’s disease

HVT (in ovo)          HVT + LAB (in ovo)          HVT (in ovo) + YFP (Feed)

Md5                      612

N/A
• All genes chosen for deoptimization were
  – Unique
  – Annotated at both the gene and protein level
  – Of manageable length for *de novo* synthesis and cloning

**MDV Codon de-optimization**

- UL19 (MCP/VP5)
- UL27 (glycoprotein B)
- UL53 (glycoprotein K)
- UL54 (ICP27/ DNA pol)
• The initial ~40 bases and the final ~40 bases were left in the wild type sequence.
• Codon pair deoptimization in UL54 significantly reduces the incidence of Marek’s disease

UL54 recoding reduces Marek’s incidence in inoculated birds

- B40 BAC: 5 trials, 67 birds
- UL54 M1: 4 trials, 60 birds
- UL54 M2: 3 trials, 48 birds
- gK M1: 1 trial, 33 birds
- UL19 M1: 1 trial, 50 birds
- gB M1: 1 trial, 50 birds

* P = 0.004
* P = 0.003
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Thank you for your attention