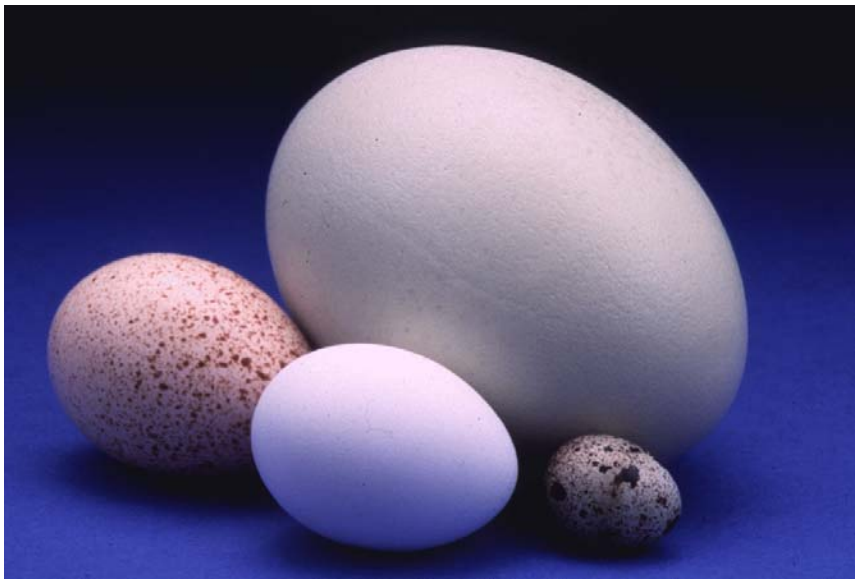
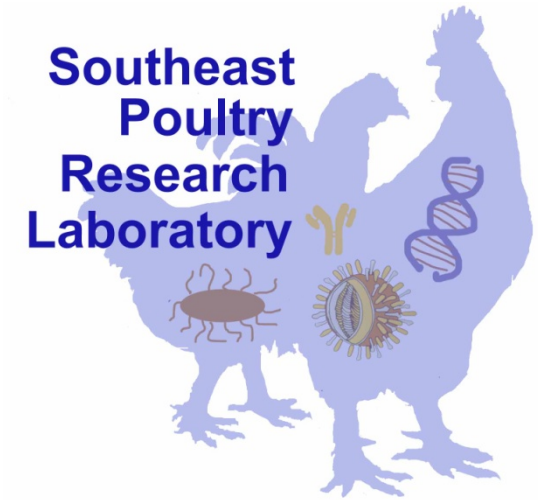
H1N1 Research Activities





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Major Accomplishments

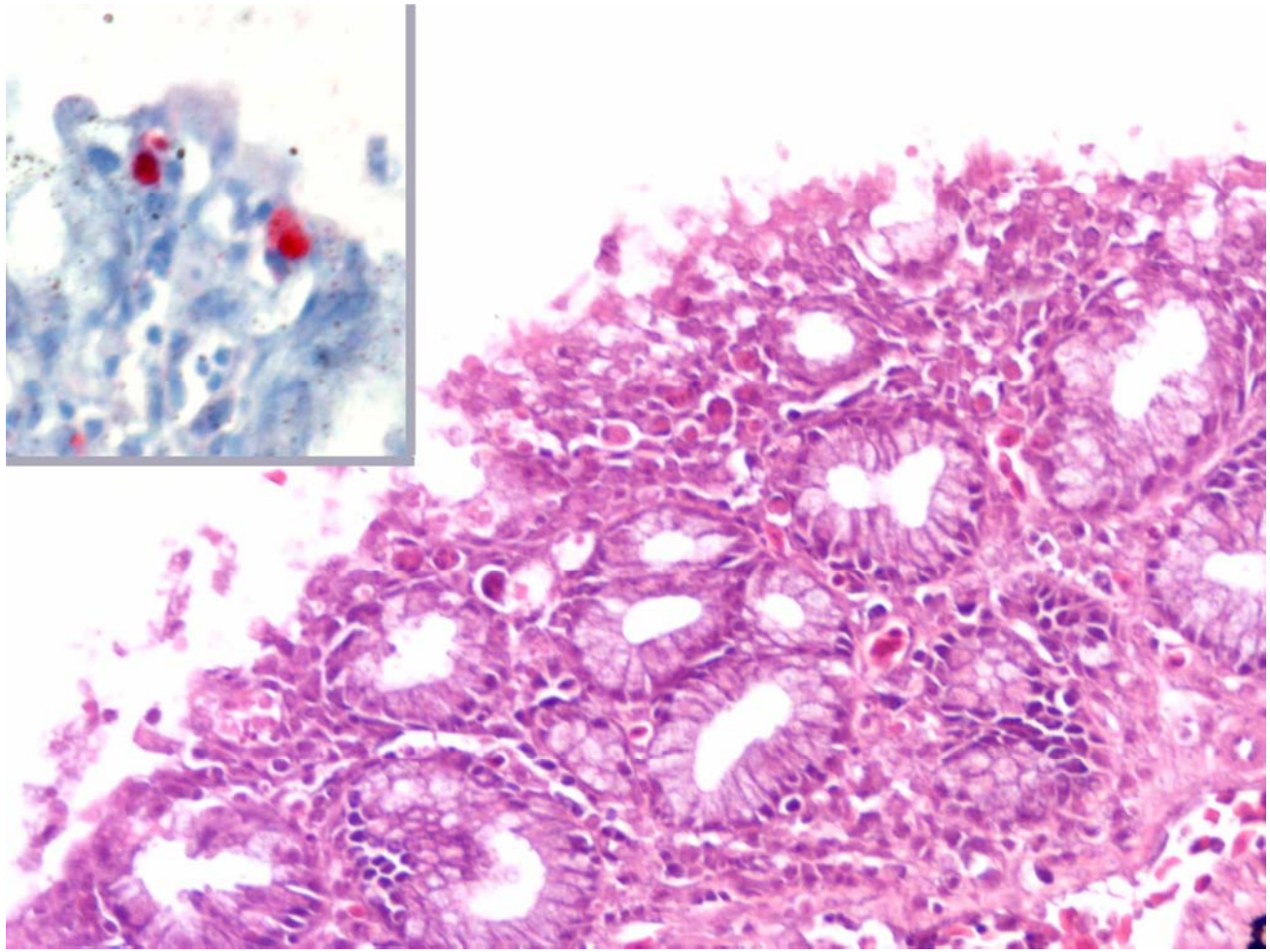
Infectivity and Pathogenicity H1N1v for Poultry

- **IVPI (chickens) = 0.00; not of high pathogenicity (A/Mexico/4108/09)**
- **Chickens**
  - **No clinical signs**
  - **No virus detected RRT-PCR or isolated from OP or CL swabs from IN-inoculated and contact-exposed birds**
  - **All HI negative at 15 DPI**
- **Quail**
  - **No clinical signs**
  - **Virus detected OP swabs at 4 DPI of IN group**
  - **IN-inoculated group = HI+, contact-exposed = HI-**

Major Accomplishments

Infectivity and Pathogenicity of H1N1v for Poultry

- **Quail – rhinitis with epithelial erosion and mucosal inflammation**



Major Accomplishments

Infectivity and Pathogenicity of H1N1v for Poultry

- **Turkey Hens and poults**
  - **No clinical signs**
  - **No virus isolated from OP or Cl swabs or tissues**
  - **HI- at 15 DPI for all groups**
- **Domestic Ducks (7 DPI)**
  - **No clinical signs**
  - **No virus detected on 2, 4 and 7 DPI**
- **Recent reports of H1N1v in Chilean Turkeys**
  - **First report in avian species**
  - **Suggests change in the virus in Chile or secondary factors that increased risk of infection (immunosuppression)**
  - **Additional laboratory studies are needed**

rRT-PCR H1N1 Tests

- **Differential test for H1N1v and Classic North American Swine H1N1 lineages targeting N1 gene**
- **Optimized for Smart Cycler 2 (Cepheid) with OneStep Kit (Qiagen) and 7500 FAST (Applied Biosystems) with AgPath ID kit (Ambion)**
- **Sensitivity:**
  - **Classic H1N1 assay: min. detection $10^{1.3\text{-}2.5}$/ml $TCID_{50}$**
  - **H1N1v assay: min. detection $10^{1.9\text{-}2.1}$/ml $TCID_{50}$**
- **Specificity:**
  - **H1N1v test only detected 2009 H1N1v viruses**
  - **Classic H1N1 test only detected N. Amer H1N1 SIV**
  - **Neither N1 test detected European H1N1 SIV, Eurasian H5N1, N. Amer. avian N1 or human seasonal N1 viruses**

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Matrix Gene Test Update

(Spackman et al. J. Clin. Microbiol. 40(9):3256-3260, 2002

- **Poor sensitivity for the 2009 Emergent H1N1 lineage observed with the USDA Type A rRT-PCR test (M gene test)**
- **Four mismatches identified in the reverse primer**

```
2009 North American  3’-cagagactggaaagtgtctttgca-5’
   2002 M-124 primer 3’-cagagacttgaagatgttttgca-5’
   2009 M-124 primer 3’-cagagactggaaagtgtctttgca-5’
```

- **Updated reverse 124 primer to be a 100% match with the 2009 Emergent H1N1 Lineage M gene sequence and optimized test with both the 2002 and 2009 reverse primers in the same reaction**
- **Initial testing showed that sensitivity was improved to the new lineage and unchanged for other lineage viruses**

Planned Studies

Infectivity and Pathogenicity of H1N1v for Poultry

- **Assessing Chilean H1N1v for infectivity for Poultry**
  - **Human H1N1v (requested of CDC)**
  - **Turkey H1N1v (requested)**
- **Species:**
  - **3 week old turkey poults**
  - **Young Japanese Quail**
- **Study:**
  - **IN, 10^6 EID_{50}**
  - **Contact transmission**
  - **Serological and virological evidence of infection**
- **Generate rg viruses to determine genetic changes responsible for infectivity in turkeys**

***Funding from USDA and CDC**

Thank You For Your Attention!