FAZD CENTER

NATIONAL CENTER FOR FOREIGN ANIMAL AND ZOONOTIC DISEASE DEFENSE

A Department of Homeland Security National Center of Excellence

# Understanding Avian Influenza Virus: Infection, Pathogenicity and Prevalence

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UPR PONCE

# Introduction

- **Influenza is a highly contagious respiratory disease, caused by influenza viruses.**
- **Wild waterfowl are considered natural reservoirs for type A influenza viruses and the source of influenza viruses (Perez, *et al*., 2003; Wan and Perez, 2006).**
- **Occasionally, Avian Influenza Viruses (AIV) are transmitted to other hosts such as pigs, horses, domestic poultry, and humans.**
- **AIV reported pandemics (1957 and 1968) have been attributed to genetic re-assortment between avian and porcine viruses (Schafer, *et al*., 1993; Ito, *et al*., 1998).**
- **In 1997 H5N1 (highly pathogenic) influenza viruses were transmitted from chickens to humans without the intervention of an intermediate host (Perez, *et al*., 2003; Wan and Perez, 2006).**

# Introduction

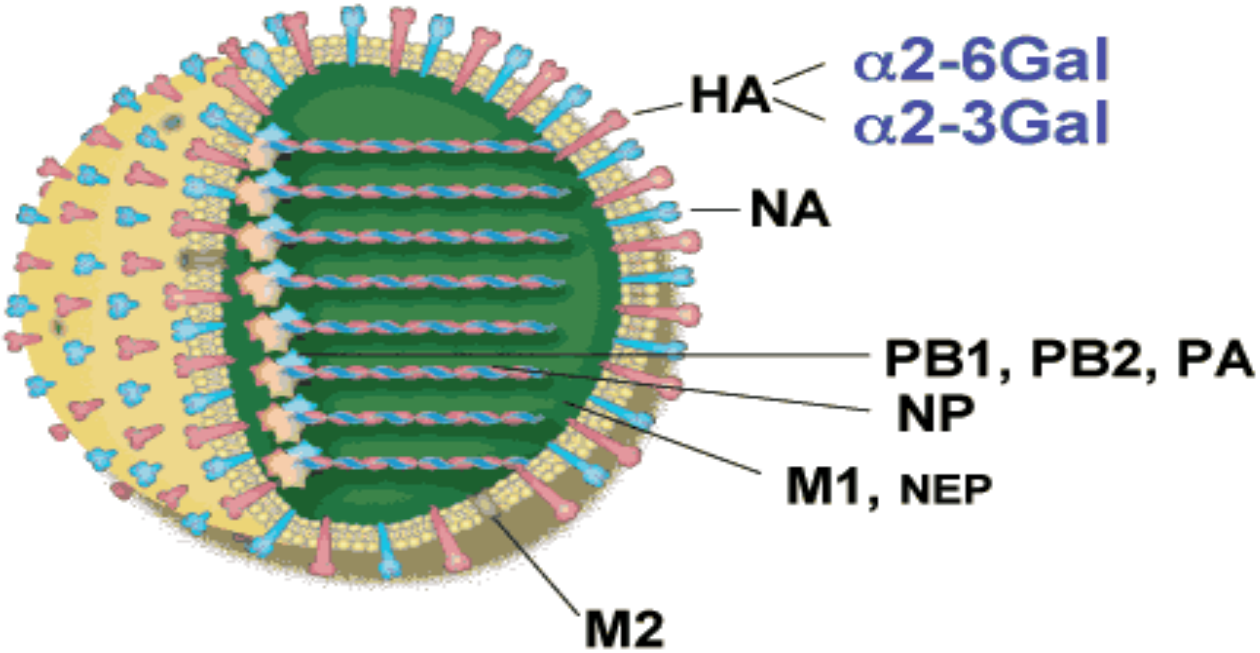
- **Influenza Virus infections are mediated by specific interactions between the viral glycoprotein hemagglutinin (HA) and the host cell oligosaccharides, which contain a terminal residue of a nine-carbon sugar named sialic acid (SA).**

- **Most AIV isolated from birds samples, bind preferentially to the *N*-acetylneuraminic acids (NanAc) that present α2-3-Gal linkages.**

- **In contrast, Influenza viruses isolated from human and swine are biased to infect receptors with NanAc expressing α2-6-Gal linkages.**

- **Receptor specificity has been postulated as to provide the most effective host barrier for crossed- influenza virus infections.**

# α2-6-Gal vs α2-3-Gal



# Research Projects

- Project 1*:

  Identification of Sialic Acid Residues from bird species
- Project 2:

  Role of HA Protein in the pathogenesis of a moderately pathogenic H5N3 AIV
- Project 3:

  AIV Surveillance System

# Project 1

# Identification of Sialic Acid Residues from bird species

# Objective

- To identify human ($\alpha$2-6) and avian ($\alpha$2-3) Sialic Acid (SA) residues on the respiratory track (trachea) of several species with the potential to act as virus reservoirs.

# Hypothesis

- Human ($\alpha$2-6) Sialic Acid (SA) residues will be present in wild species.

# Methodology

Jungle Fowl

Chicken

Rio Grande Turkey

White turkey

Bobwhite quail

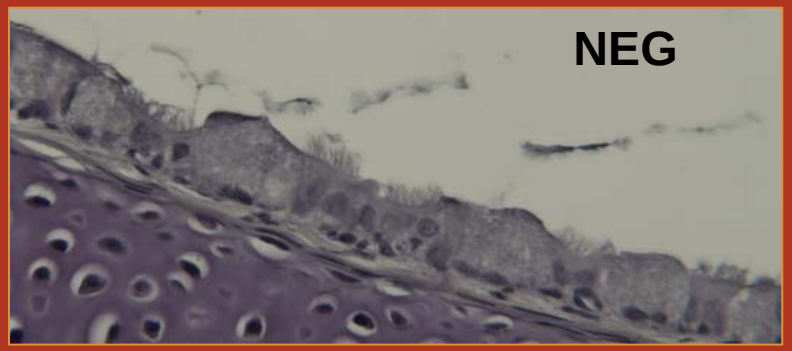
# Methodology

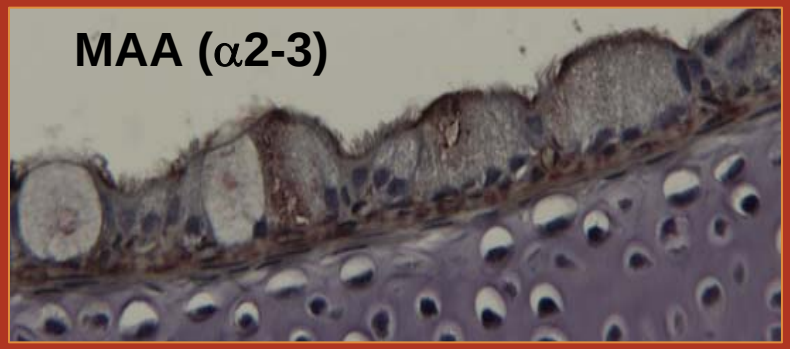
- Immunohisto-staining
  - Sialic acid linkage
    - Biotin labeled SNA lectin: ($\alpha$2-6)
      - DAB,VIP
    - Biotin labeled MAA lectin: ($\alpha$2-3)
      - DAB,VIP
  - Tubulin in ciliated cells at the respiratory tract
    - SG Staining
- Identification of tissue expression by microscopy

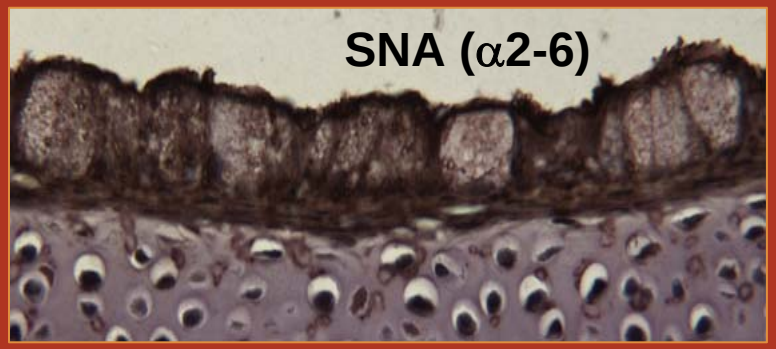
# Chicken Trachea

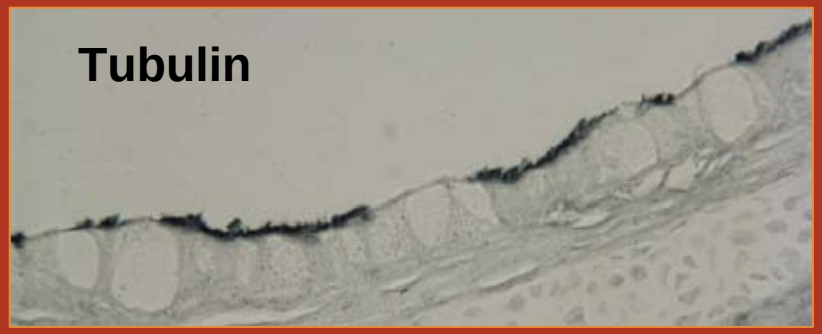
# Results:

Chicken trachea slide; DAB Staining & SG





# Results: Summary

| Species | Human ($\alpha$2-6) | Avian ($\alpha$2-3) |
|---|---|---|
| **Chicken** | + | + |
| **Bobwhite quail** | + | + |
| **Pig** | + | + |
| **White Turkey** | - | + |
| **Jungle Fowl** | + | - |
| **RG Wild Turkey** | + | + |

# Conclusion

The results indicated the presence of both human and avian specific receptors in the chicken, bobwhite quail, pig, and Rio Grande turkey. These findings suggested that these hosts may play an important role in AIV inter-species transmission.

# Project 2

# Role of HA Protein in the pathogenesis of a moderately pathogenic H5N3 AIV

# Objective

- To study the influence of AIV-mutants in the pathogenesis of the virus by means of the host immunological response.

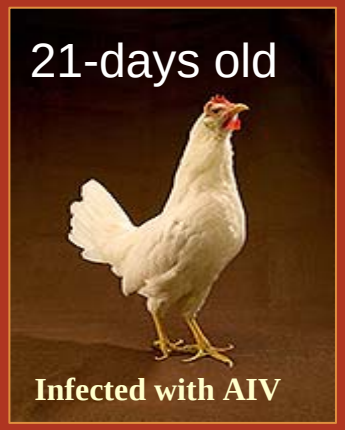
# Methodology

SPFL White Leghorn

1 day-old

Observed

Grown




Observed

Observed

**7 groups of 18 each:**

4 days

N=9

7 days

N=9

# Results

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Positive control (0.124) | Normal sera (0.059) | Positive control (0.177) | 8 (0.079) [0.013] {0.206} | 9 (0.092) [0.036] {0.363} | | | [0.026] {0.196} | [0.023] {0.257} | | | |
| B | 1008 (0.06) [-0.001] {0.003} | 1010 (0.061) [0.001] {0.01} | 1013 (0.062) [0.006] {0.002} | 1014 (0.061) [-0.005] {0.046} | 1015 (0.061) [-0.006] {0.008} | 1016 (0.059) [-0.006] {0.117} | 1017 (0.081) [0.004] {0.119} | 1022 (0.064) [-0.007] {0.054} | 1023 (0.119) [0.085] {0.271} HT | 1026 (0.100) [0] {0.149} | 1028 (0.088) [0.039] {0.081} | 1029 (0.061) [-0.009] {-0.002} |
| C | 1030 (0.059) [-0.007] {0} | 1031 (0.06) [-0.004] {0.01} SHL | 1032 (0.06) [0.001] {0.004} SHL | 1035 (0.069) [0.26] {0.117} | 1037 (0.106) [0.1] {0.205} | 1039 (0.084) [0.005] {0.317} | 1040 (0.076) [0.018] {0.179} SHL | 1043 (0.09) [0.012] {0.119} | 1045 (0.059) [-0.008] {0.009} | 1046 (0.06) [-0.008] {0.01} | 1047 (0.58) [-0.005] {-0.001} | 1048 (0.058) [0.007] {0.001} |
| D | 1049 (0.076) [0.014] {0.108} | 1050 (0.068) [-0.005] {0.122} | 1051 (0.066) [0.014] {0.085} | 1053 (0.083) [0.007] {0.161} HL | 1054 (0.073) [-0.005] {0.171} | 1061 (0.062) [-0.008] {0.013} | 1062 (0.061) [-0.004] {0.045} | 1063 (0.061) [-0.002] {0.02} HT | 1064 (0.058) [-0.006] {0.031} | 1065 (0.091) [0.013] {0.162} | 1068 (0.111) [0.008] {0.286} | 1070 (0.079) [0.033] {0.137} |
| E | 1073 (0.072) [0] {0.161} | | | | | | | | | | | |
| F | 1096 (0.06) [-0.002] {0.007} HL | 1097 (0.07) [0.019] {0.092} | 1098 (0.06) [-0.002] {0.053} | 1084 (0.084) [0.055] {0.295} | No band (0.059) [-0.006] {0.031} | 1 (0.058) [0.008] {0.013} | 2 (0.06) [-0.007] {0.015} | 3 (0.059) [0] {0.01} | 4 (0.058) [-0.009] {0.015} | 5 (0.061) [-0.007] {0.014} | 6 (0.059) [-0.009] {0.001} | 7 (0.061) [-0.007] {0.001} |
| G | 1018 (0.06) [-0.008] {0.001} | 1019 (0.058) [-0.009] {-0.001} | 1020 (0.058) [-0.004] {0.004} | 1021 (0.057) [-0.007] {0.006} | 1024 (0.058) [-0.006] {0.011} | 1025 (0.06) [0.001] {0.012} | 1027 (0.059) [-0.008] {0.004} | 1033 (0.058) [-0.007] {0.008} | 1034 (0.058) [-0.005] {0.007} | 1038 (0.061) [-0.003] {0.011} | 1041 (0.06) [-0.009] {-0.003} | 1042 (0.059) [-0.008] {0} |
| H | 1044 (0.057) [-0.009] {-0.002} | 1052 (0.057) [-0.006] {0.002} | 1055 (0.073) [0.003] {0.014} | 1056 (0.057) [0.002] {0.013} HL* | 1057 (0.058) [0] {0.016} HL | 1058 (0.058) [-0.002] {0.013} | 1059 (0.066) [0.001] {0.024} | 1060 (0.059) [-0.002] {0.015} NL | 1100 (0.058) [-0.004] {0.008} | Normal sera (0.058) | Positive control (0.222) | Normal sera (0.059) |

PBS/controls 102 118 119 116 113 103

# Conclusion

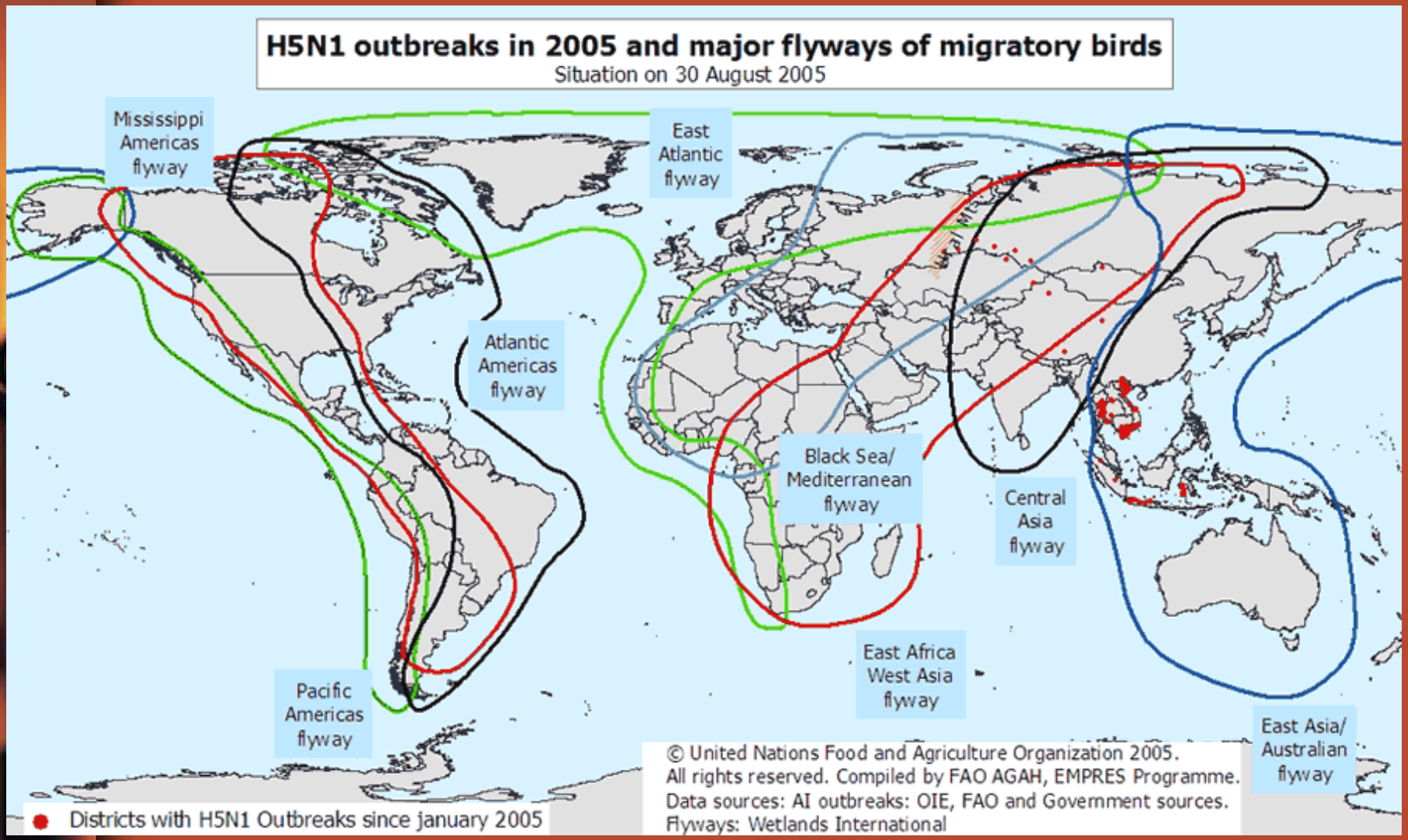
- Some AVI virus strains are more pathogenic than others as it is the case if the 113 mutant.

# Project 3

# AIV Surveillance System

# Objective

- To develop an early detection system for AIV in the wildlife, in order to prevent the introduction and adaptation of the virus to other hosts, including humans.


H5N1 outbreaks in 2005 and major flyways of migratory birds
Situation on 30 August 2005
Mississippi Americas flyway
East Atlantic flyway
Atlantic Americas flyway
Black Sea/ Mediterranean flyway
Central Asia flyway
East Africa West Asia flyway
Pacific Americas flyway
East Asia/ Australian flyway
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Data sources: AI outbreaks: OIE, FAO and Government sources.
Flyways: Wetlands International
Districts with H5N1 Outbreaks since january 2005

# Programa de Vigilancia de *Influenza aviar*

***Dra. Edu B Suarez***

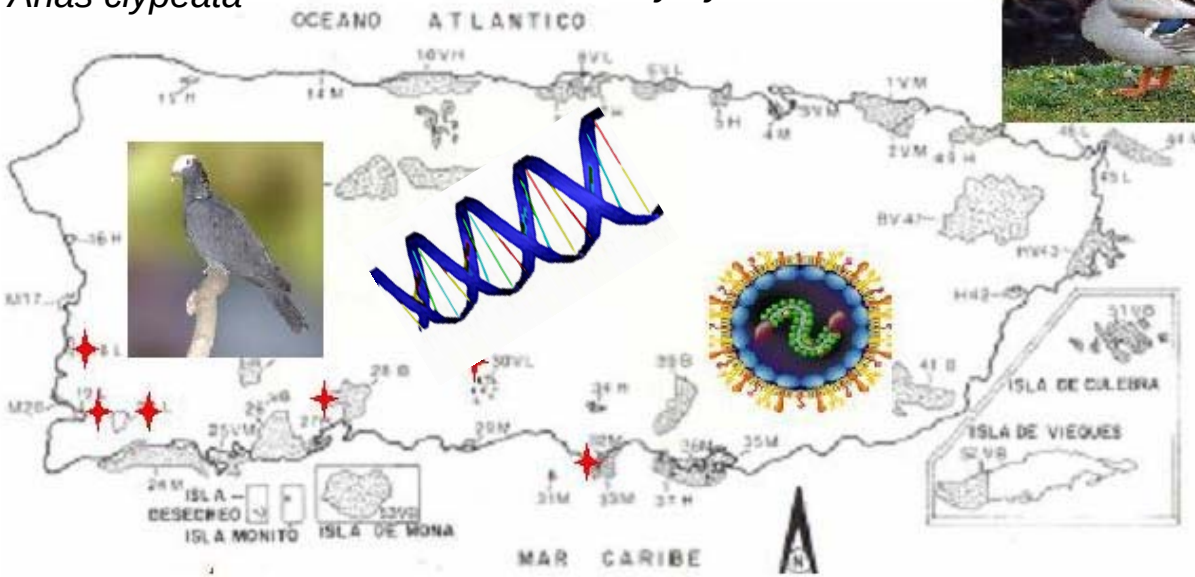
*Anas crecca*

*Anas clypeata*

*Aythya affinis*

*Anas platyrhynchos*

*Aythya collaris*



*Anas americana*

*Anas acuta*

*Fulica Americana*

*Dendrocygna bicolor*

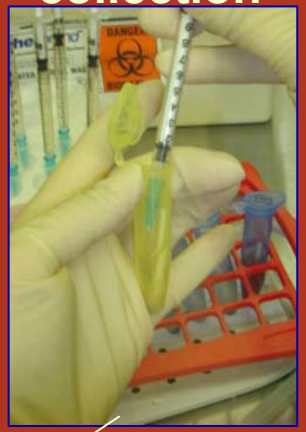
*Anas discors*

# Methodology

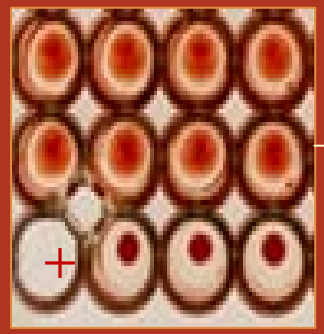
**Sample collection**

**9 days- chicken embryo Egg Inoculation**

**Allantoic fluid collection**



**Hemagglutination Test**




**Hemagglutination Inhibition Test**

**AIV FluDetect**

# Future Projections

- To identify the presence of avian and human specific receptor in other species with the potential of serving as virus reservoirs
- Develop a wildlife Surveillance Program in Puerto Rico.

# Integrated Strategies for increasing Understanding, Preparedness, Response, and Research in Terrorism at the UPR-Ponce

DHS Scientific Leadership Award for Minority Serving Institutions
(2008-ST-062-000001).

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DEPARTAMENTO DE RECURSOS NATURALES Y AMBIENTALES

88

92
91

InstaGard PL
Powdered Latex Exam Gloves
95

102

120

# Other Activities

- Microscope Training
  - Dr. John Roth (June 25)
- Ambion, Austin
  - Dr. John El-Attrrache (June 27)
  - RNA Extraction
  - NAHLN reference
- BioTrove
  - Dr. Matt Lawes (June 28)
- Hi-Line (July 12)
- T A & M Vet Diagnostic Lab. In Gonzales
  - Dr. Linares (July 23)
  - Necropsy

# Impact of DHS MSI Programs:

- Contrib ted activitie

- Suppor

# Acknowledgements


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- Dr. Thomas Powdrill
- Dr. Dave Pledger
- DHS Summer Research Team Program for Minority Serving Institutions

GONZALES
FOOD MKT.
HOT SAUSAGE
BAR-B-Q & BEER