

The background of the slide is a painting of several ducks in a body of water. One duck is in the foreground on the left, facing left. Another duck is in the center, facing right. A third duck is on the right, facing right. In the background, a person is visible in the water. The painting is in a soft, painterly style with muted colors.

SURVEILLANCE OF AVIAN INFLUENZA IN SOUTH AFRICA:

**A LOOK AT A ZOOLOGICAL MONITORING PROGRAM
AND SAMPLING OF WILD BIRDS**

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About Me

- B.S. Biology (2006)
- Beginning my second year as a veterinary student
- One more requirement for the MPH degree (disease epi)



Where we are going...

- Avian Influenza and why it is important
- Monitoring Program at the National Zoological Gardens of South Africa
- Wild Bird Capture and Sampling in Cape Town
- Diagnostics of Samples

Where I have been.



Avian Influenza

- Type A influenza of the family *Orthomyxoviridae*
- Found primarily in wild birds.
- RNA virus
- Categorized as either low pathogenic avian influenza (LPAI) or high pathogenic avian influenza (HPAI).
- Carried in saliva, nasal secretions, and feces of infected birds.
- Also spread by contaminated surfaces
- Incubation period ranges from 2-8 days
- Symptoms – diarrhea, respiratory distress, weakness, picking/scratching at themselves, poor appetite, and other behavior abnormalities .
- Usually stays true to the species it normally uses as a host.

History of Disease

- Ten outbreaks have caused illness in humans
 - H₅N₁, H₇N₃, H₇N₇, and H₉N₂
- Pandemic of 1918
 - 1/3 of the world's population was infected
 - caused 675,000 deaths in the United States and 20-50 million deaths worldwide.
 - No strain was isolated but all descendants cause milder disease
 - the 1918 virus is the likely ancestor of all 4 of the human and swine H₁N₁ and H₃N₂ lineages, as well as the "extinct" H₂N₂ lineage
- 1957: Asian Influenza
 - H₂N₂
 - 1 million deaths globally
- 1968: Hong Kong
 - 1 million died
 - H₃N₂

Current Avian Outbreak of H5N1

- First discovered in China, 1996.
- Can now be found in Asia, Europe, the Near East, and in the northern countries of Africa.
 - endemic in many areas of these countries
 - Transmission to people limited and sporadic.
 - Mortality rates are very high.
 - 2003 to June 19, 2008
 - 385 reported cases of H5N1 infections in humans worldwide and 243 of these have died.

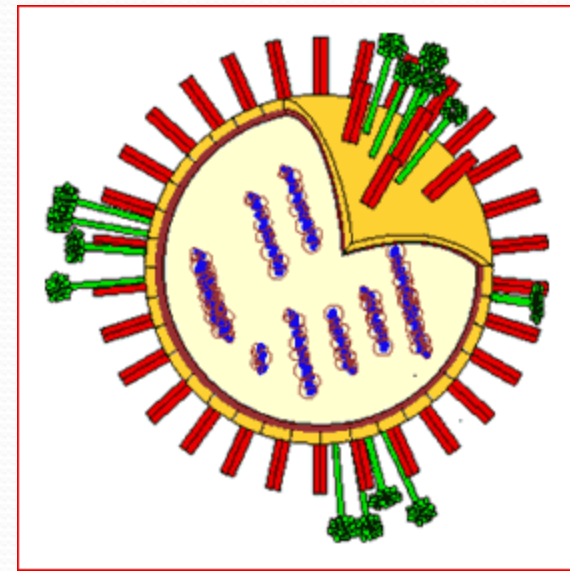
[World Map](#)

Pandemic

- Three requirements to start a human pandemic;
 - 1) A viral strain for which there is little or no immunity in humans must emerge
 - 2) Has to be able to cause serious illness in humans
 - 3) Has to spread easily from humans to humans.
- The H5N1 strain has the first two of the three requirements; it has not successfully transferred from person to person.
- Two methods for increasing transmissibility among humans;
 - Reassortment -genetic material is exchanged between human and avian viruses by having co-infection of a human or a pig.
 - Adaptive mutation- slower process where the virus has the capability to bind to human cells after subsequent infections of humans .

Molecular Level

- HA receptor-binding site configuration is different for influenza viruses adapted to infect birds and those adapted to infect humans.
- Strains adapted to birds bind sialic acid receptors with α (2-3) linked sugars.
- Human-adapted influenza viruses bind receptors with α (2-6) linkages.
- The switch from this avian receptor configuration requires of the virus only 1 amino acid change.



Pandemic in Africa

- Africa has a unique population
- Major issues with HIV/AIDS, tuberculosis, and malaria
- Further complications and more severe infections of avian influenza are likely to arise due to the presence of many immune compromised individuals



Recent News

- H₉N₂ new possible source of an avian influenza pandemic.
- Strain is increasing in prevalence.
- Caused illness in at least four children in Hong Kong
- Can be found in humans, poultry, and pigs.
- Mixed with H₃N₂ (common cold) to show its ability for reassortment
- Not found to be transmitted via aerosol, but can still be spread through contact with fomites.

Avian Influenza in South Africa

Three occasions of HPAI

- 1. 1963: 1,300 common terns died off the coast of the Western Cape.
 - H5N3 was isolated--first isolation of the AIV in wild birds in South Africa.
- 2. 2004: H5N2 detected in ostriches of Eastern Cape Province.
 - First detected when the mortality rate suddenly increased from 5% to 44% over five months.
 - 26,000 ostriches culled
 - At same time, LPAI H5N2 was isolated in wild Egyptian geese of the Western Cape. Suggests the virus originated in wild birds of Western Cape and may have mutated into a highly virulent form in ostriches of the Eastern Cape and also some ostriches of the Western Cape.
- 3. 2006: reemergence of HPAI H5N2 in ostriches
 - Although the two strains of 2004 and 2006 shared a common ancestor, they were not related.

Why outbreaks are so rare here

- Factors attributing to this fact:
 - No large-scale duck or turkey farming in the area
 - Although ostriches may be periodically infected, they are atypical terrestrial hosts.
 - Ostrich farms and the main poultry producing areas are geographically separated
 - There is such a dry climate most of the year that many of the water birds remain congregated around large bodies of water.

National Zoological Gardens of South Africa



Zoos as a Sentinel for Avian Influenza

- Have the facilities, staff, lab equipment, and medical history on all of the birds in their collection
- Promote interaction and close proximity between a variety of animals and humans, including staff, veterinarians, and the public
- Dr. Tracey McNamara from the Bronx Zoo, West Nile Virus
- House very specific species of birds
 - threatened or endangered
- More natural looking habitats.
- Airport confiscation and illegal exotic bird trade

Writing an SOP for a zoo

- Took into account four different scenarios with increasing severity; current preventative measures, an outbreak of avian HPAI in South Africa, an outbreak of avian HPAI at the National Zoo, and in the event of a human pandemic of HPAI in South Africa

Factors to Consider

- Surveillance Program
- When to Close the zoo?
- Personnel Safety
- Impact on workers
- Proximity of target species
- Feeding raw poultry
- Facilities for quarantine
- Pathology Facilities
- Confiscated animals
- Farm Park Poultry
- Walk through aviary
- Disposal of carcasses
- Disinfection

NZG Sampling Program

- 94 birds sampled
- Opportunistic Sampling
- Winter Monitoring (May-August)



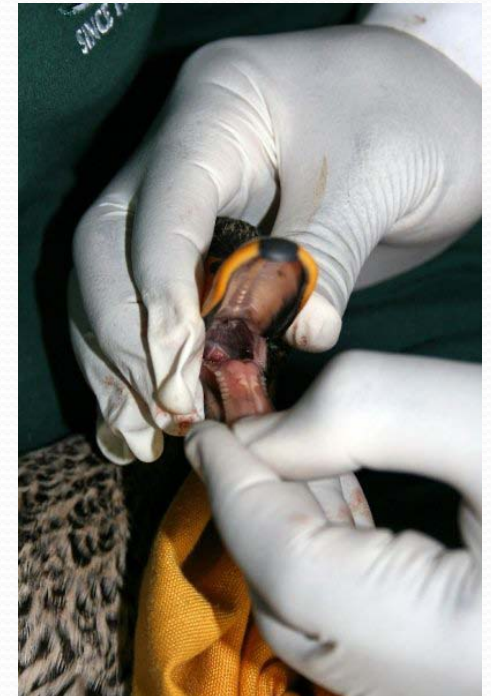
	Collection Date	Species	Sex	Live Bird	Necropsy	Choana	Cloaca	Date Taken to OVI
1	14.05.08	Cape Vulture	0.0.1		08/264	X	X	
2	15.05.08	Parrot	0.1		08/268	X	X	
3	21.05.08	King Vulture	0.1	Chick 1/08		X	X	
4	21.05.08	King Vulture	0.0.1	Chick 2/08		X	X	
5	21.05.08	King Vulture	0.0.1	Chick 3/08		X	X	21.05.08
6	22.05.08	Bald Ibis	0.0.1	PTA Zoo 14-01		X	X	
7	22.05.08	Bald Ibis	1.0		08/273	X	X	
8	27.05.08	Hadedda Ibis	0.1	Free Range		X	X	
9	24.05.05	Goliath Heron	1.0		08/251	X	X	
10	26.05.08	Rosy Flamingo	1.0		08/280	X	X	
11	26.05.08	Egyptian Goose	0.1		08/275	X	X	29.05.08
12	29.05.08	Giant Eagle Owl	1.0		08/290	X	X	
13	29.05.08	Brown Headed Parrot	0.0.1		08/288	X	X	
14	29.05.08	Black Swan	0.01		08/289	X	X	
15	30.05.08	Black Swan	0.0.1	Donation		X	X	

Demographics

- 94 birds
- Species:
 - Vultures, Parrots, Ibis, Herons, Flamingoes, Herons, Geese, Owls, Egrets, Macaws, Conures, Doves, Cranes, Lorikeets, Corellas, and more.
- 32 birds sampled during necropsy
- 62 live birds sampled

Swabbing

- Choanal and Cloacal Swabs
- Invasive sterile collection swab with a wire shaft
- Phosphate buffered saline to be frozen at 4 degrees Celsius



Postmortems

- Microscopic and histopathological evidence
- Necessary for discerning patterns on why groups of species may be dying off
- Precautionary measures: Ventilation hood.
 - All birds
 - Air-borne chlamydial infection
- Clean necropsy report is not sufficient evidence for a negative bird.
- Symptoms of avian influenza would present with increased nasal discharge, decrease in overall condition, a heavily soiled cloacal region, pulmonary consolidation, and possibly multifocal hemorrhage in the organs



Wild Bird Capture



Disease detection in the wild

- Increased abundance of dead or dying birds
- Postmortems and pattern recognition
- Challenges
 - Birds that die of illness are quickly assimilated by the environment
 - skews data on disease prevalence
 - Search for sick or dead animals subject to bias
 - large and colorful birds are found more easily than those that blend into their surroundings



GAINS

Global Avian Influenza Network for Surveillance

- Set up to expand operational field capabilities, improve the understanding of viral strains and transmission of influenza viruses in wild birds, and to disseminate information to all levels of governments, international organizations, the private sector and the general public through regular sampling of wild birds and also consistent sharing of data through a globally accessible data bank.
- One common database

One effort of GAINS

- Five different sites, two of which are in South Africa, one in Botswana, one in Zimbabwe, and one in Mozambique
- Each location is visited every two months to count, catch, and process birds.

Cape Flats Water Treatment Plant in Strandfontein

- 15 ponds and several channel-ways. This is a prime location for surveillance of disease based on many factors.
 - series of ponds located between a river and the False Bay, which allows for a diverse collection of species.
 - Unique connection to humans since this is a water treatment facility very close to civilization.



Do all birds matter?

- One of the key wild vectors of avian influenza, are all of the duck species.
- Also on the rise as a commonly infected wild bird species are the swans

The Mute Swan

- Swans are on the rise as a commonly infected wild bird species
- The mute swan is the most likely swan species to transmit HPAI.
- Experimentally infected swans
- Discovered that they are highly susceptible and can be clinically protected by pre-exposure immunity.



South African Birds

- Less predictable fly pattern than the northern hemisphere
 - Variable amounts of rainfall each year and generally warm climate.
- Birds find safety in water and during drier seasons can become subject to the many predators encompassing Africa
 - take up residence on the larger bodies of water that are at less risk of running dry.
- The ducks are opportunistic migrators in order to evade predation, to breed, or to molt. For this reason, it becomes more of a challenge to ascertain the movement of sick birds
- Ducks found in the Cape region have not been recorded as flying any farther north than Tanzania.

Census of Wild Birds

- Essential for gaining an understanding about the population of the area.
- Count for 30 minute intervals for all species of birds that come within 150 meters.
- Interaction with the environment is noted
- Water quality is routinely measured in all of the ponds.

Capture of Wild Birds

- Traps were set at different ponds and also along three channel-ways.
- Two main types of traps used, walk-in traps and mist nets.



Processing Birds

- 49 birds were collected and processed.
- Species captured included Egyptian geese, yellow billed ducks, cape teals, red eyed doves, speckled pigeons, cape wagtails, cape shovelers, and cape gulls.
- Processing includes:
 - ringing the birds for future identification, taking morphometrics, examining the health status, collecting feather samples, drawing blood, taking photographs, and swabbing for avian influenza.
- After the birds have been processed, the ones that are not molting were released on site. The birds that were undergoing different stages of molt were taken back to their capture location for release.





Diagnostics

Viral Isolation

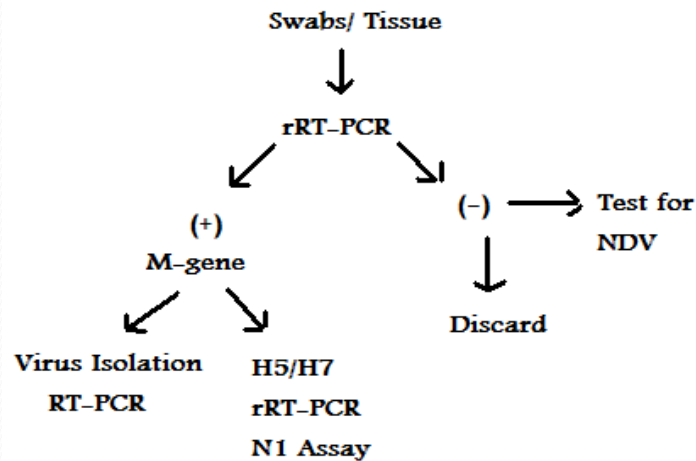
MagNA Pure

automated machine
Large batch samples

Trizol

Preferred, slower
Individual samples

rRT-PCR



Results

- Fifteen of the ninety four birds showed signs of being suspect positive for avian influenza M gene
- but with further typing, no H5/H7 gene could be extracted and no other virus isolation was found.

Suspect Positives

Species	Live Bird	Necropsy	Sample Type	H5/H7 gene rRT-PCR	Virus Isolation
Cape Vulture		08/264	Oropharyngeal Swab	Negative	Negative
King Vulture	Chick 1/08		Oropharyngeal Swab	Negative	Negative
King Vulture	Chick 2/08		Cloacal Swab	Negative	Negative
Bald Ibis	PTA Zoo 14-01		Oropharyngeal Swab	Negative	Negative
Bald Ibis	PTA Zoo 14-01		Cloacal Swab	Negative	Negative
Bald Ibis		08/273	Oropharyngeal Swab	Negative	Negative
Spoonbill Chick		08/330	Oropharyngeal Swab	Negative	Negative
Coco Lead Beater		08/328	Cloacal Swab	Negative	Negative
Red Eyed Dove	Bird 5		Cloacal Swab	Negative	Negative
Red Eyed Dove	Bird 6		Oropharyngeal Swab	Negative	Negative
Little Corella		08/400	Cloacal Swab	Negative	Negative
Little Corella		08/400	Cloacal Swab	Negative	Negative
Red Sided Parrot		08/394	Oropharyngeal Swab	Negative	Negative
Red Sided Parrot		08/394	Cloacal Swab	Negative	Negative
Laughing Dove	Bird 3		Oropharyngeal Swab	Negative	Negative
Little Corella		08/401	Oropharyngeal Swab	Negative	Negative

Discussion

- No conclusive evidence that there is any strain of avian influenza in the birds sampled.
- Other factors could have been weakening in the primers as the PCR cycle comes to an end.
- Prevalence of suspect positive birds for avian influenza is 14.13%
- Both the oropharyngeal and the cloacal swabs were useful in detecting the virus so one should not be favored over the other especially since only three of the suspect positive birds had both swabs pick up signs of a virus.
- No association between more live birds or postmortem birds sampled showing signs of the virus
- Future research at the zoo could include sampling of more water birds or ratites such as ostriches

“Other” Public Health Exposure



RINGWORM!!!!



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Questions?

