SURVEILLANCE OF AVIAN INFLUENZA IN SOUTH AFRICA:
A LOOK AT A ZOOLOGICAL MONITORING PROGRAM AND SAMPLING OF WILD BIRDS

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MPH Presentation
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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 *Orthomyoxociridae*
- 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 descendents 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]
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 $\alpha (2\,\rightarrow\,3)$ linked sugars.
- Human-adapted influenza viruses bind receptors with $\alpha (2\,\rightarrow\,6)$ linkages.
- The switch from this avian receptor configuration requires 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)
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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.
<table>
<thead>
<tr>
<th>Species</th>
<th>Live Bird</th>
<th>Necropsy</th>
<th>Sample Type</th>
<th>H5/H7 gene rRT-PCR</th>
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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!!!!
References


Questions?