Experiences of the early detection and treatment of aspergillosis in falcons in the Middle East

- Antonio Di Somma
- Dubai Falcon Hospital
- Tom Bailey
- International Wildlife Consultants
Dubai Falcon Hospital

- Established by HH Sheikh Hamdan bin Rashid al Maktoum, Deputy Ruler of Dubai, in 1983
- 1st falcon hospital in region
- 2,000 falcons/ year
- Externship training programme
- Service for local wildlife collections
International Wildlife Consultants

Falcon breeding and research facility
Breeding falcons since 1975
Field projects in Central Asia region
International Festival of Falconry

Established by Dr Nick Fox, a raptor biologist
Based on a 278 acre farm in Wales
22% of farm out of agriculture Tir Gofal
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td>Welcome ceremony</td>
<td>HE Mohammed Al Bowardi</td>
</tr>
<tr>
<td>10:00</td>
<td>Hall A: Raptor Health, First Aid and Rehabilitation</td>
<td></td>
</tr>
<tr>
<td>10:20</td>
<td>1. Novel Therapeutic Agents and Treatment Modalities for Falcons</td>
<td>Dirk Verwoerd &amp; Tom Bailey</td>
</tr>
<tr>
<td>10:40</td>
<td>2. What Falcons Need to Know About the Pre-purchase Veterinary Examination of Falcons</td>
<td>Dr. Tom Bailey</td>
</tr>
<tr>
<td>11:00</td>
<td>Morning Tea Break</td>
<td></td>
</tr>
<tr>
<td>11:30</td>
<td>3. Traditional Arabian Falcon Remedies, History and Significance</td>
<td>Dr. Tarik Al-Tarabendi</td>
</tr>
<tr>
<td>12:00</td>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td>12:30</td>
<td>4. Veterinarian’s Role in Raptor Breeding Programmes: It’s Worth It</td>
<td>Marino Garcia</td>
</tr>
<tr>
<td>13:00</td>
<td>5. Risk and Prevention of Travel-related Disease in Falconry Birds</td>
<td>Dr. Pat Redig</td>
</tr>
<tr>
<td>12:45</td>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td>13:45</td>
<td>6. The Green Balkans Raptor Rehabilitation Centre</td>
<td>Izyaiko Kostrovic</td>
</tr>
<tr>
<td>14:00</td>
<td>Morning Tea Break</td>
<td></td>
</tr>
<tr>
<td>14:30</td>
<td>7. Vulture Crisis in India</td>
<td>Dr. Vishu Prakash</td>
</tr>
<tr>
<td>14:40</td>
<td>8. Update on the Treatment of Aspergillosis in Falcons</td>
<td>Dr. Antonio Di Somma</td>
</tr>
<tr>
<td>14:50</td>
<td>9. Rehabilitating Falcons in the UAE - Experience at Sharjah Wildlife Centre</td>
<td>Dr. An Pas</td>
</tr>
<tr>
<td>15:15</td>
<td>10. Thoughts on the Rehabilitation and Release of Peregrine Falcons in the UK</td>
<td>Dr. Gordon Mellor</td>
</tr>
<tr>
<td>15:40</td>
<td>Close</td>
<td></td>
</tr>
<tr>
<td>16:00</td>
<td>Practical First Aid for Falcons’ Births to be held at Conference</td>
<td>Dr. Margaret Muller</td>
</tr>
</tbody>
</table>
Overview

1. Aspergillosis in raptors
2. Stress factors and disease
3. Early diagnosis (pre-purchase exams)
4. Treatment including voriconazole & update on posaconazole
5. Prevention
Aspergillosis in raptors

- Caused by fungus *Aspergillus*
- Spores ubiquitous in environment
- Spores inhaled & colonise avian respiratory system
  - warm moist airsacs
- Birds with good immune systems less susceptible
- Birds with poor immune systems susceptible
- Most significant disease of hunting falcons in the UAE
Stress

- An important factor in predisposing raptors to aspergillosis

- Defined as the sum of biological reactions to any adverse stimulus that disturbs the homeostasis of an organism.
What Causes Stress in Raptors?

- Transport
- Import and export
- Change of location
- Early stages of training
- Excessive temperatures
- Bad handling
- Treatment
- Other diseases
- Nutrition
- Breeding
Why is Stress Bad for Raptors?

- Stressed birds release a hormone called cortisol
- Cortisol depresses the immune system
- Prolonged stress results in a long term depression of the immune system
Stressors in captive-raised falcons

Stressors that can precipitate disease

- Psychological transition
- Environmental transition
- Pathogen exposure
The Breeding Facility

International Wildlife Consultants (UK) Ltd. is one of the oldest established falcon breeding facilities in Europe, having bred falcons since 1975 and exporting to the Middle East since 1987. IWC (UK) was the first falcon facility in the UK to qualify as a CITES registered breeder. We have supplied the Royal Families of the United Arab Emirates and the Royal Family of Bahrain for 20 years. Many falcons have been sent back for breeding. Our bloodlines include proven stock retired from the royal hunting falcons.

We have more than 150 breeding chambers producing some 300 top-quality, young falcons each year. Our breeding stock includes 100 pure gyrfalcon falcons from some of the finest blood lines and genetics worldwide. Particular attention has been given to bloodlines specific to white and black gyrs. We hold DNA data from all falcons at IWC. This enables straightforward and uncontroversial identification of each falcon, as well as providing a database of relatedness between individuals. On request we are able to provide a full pedigree showing parentage and direct ancestors.
Early stages of training
Temperature variation

Carmarthen, Wales

September

Avg Low: 11°
Avg High: 18°
Avg precip: 7.54 cm

Dubai, UAE

September

Avg Low: 28°
Avg High: 39°
Avg precip: 0 cm

Source: http://www.climate-charts.com/
Humidity variation

- Incidence of aspergillosis very low in Riyadh, KSA
  - Intense dry heat
- In 8 yrs Jaime Samour saw 6 cases, most from coastal areas (Jeddah)
- Incidence of aspergillosis in Dubai, UAE very high
  - Intense, humid heat
- At DFH we could see > 12 cases/day.
# Humidity variation

<table>
<thead>
<tr>
<th>NOAA Code</th>
<th>Statistic</th>
<th>Units</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riyadh</td>
<td>Relative Humidity Mean Value</td>
<td>%</td>
<td>50</td>
<td>40</td>
<td>35</td>
<td>33</td>
<td>22</td>
<td>14</td>
<td>15</td>
<td>14</td>
<td>18</td>
<td>24</td>
<td>37</td>
<td>46</td>
<td>29</td>
</tr>
<tr>
<td>Dubai</td>
<td>Relative Humidity Mean Value</td>
<td>%</td>
<td>65</td>
<td>65</td>
<td>63</td>
<td>55</td>
<td>53</td>
<td>58</td>
<td>56</td>
<td>57</td>
<td>60</td>
<td>60</td>
<td>61</td>
<td>64</td>
<td>60</td>
</tr>
</tbody>
</table>


Humidity is a term for the amount of water vapour in the air. Relative humidity is used in weather forecasts.
Humidity hits 100% in UAE; motorists warned
Uncomfortable conditions to continue on Thursday; Discomfort index touches six

By Staff
Published Thursday, August 11, 2011

Humidity hit 100 per cent in the western coastal areas of the country Wednesday, meteorologists said, urging motorists to be extra cautious.
Historical Aspergillosis

- Accounts of an incurable disease of the lungs called ‘the pantas’ by the falconer Blome in 1683
  “Falcons that are from bad methods of transportation will give evidence of fatigue when flying”
- Frederick II (1247)
Effects of Fungal Infections on The Body

- **Fungi incite immune responses:**
  - Fungi ingested by defence cells (macrophages)
  - Activated macrophages cause tissue damage:
    - release oxygen free radicals
    - release inflammatory mediators (e.g. interleukin-1)

- **Aspergillus** produce toxins:
  - Aflotoxins cause liver necrosis
  - Gliotoxin (*A. fumigatus*) suppress the immune system
  - Toxins increase susceptibility to other conditions

Wernery, U. *Aspergillus fumigatus* - a toxin producer. Falco 2003; 21; 15
Fig. 8.173 In one actual case of acute aspergillosis, a gyrfalcon (*Falco rusticolus*) was housed in a chamber adjacent to another in which a pointer dog was kept, separated by a short divider. A few days after straw was spread on the floor for the dog, the falcon succumbed to acute aspergillosis.
Clinical Aspergillosis

- Clinical signs
  - Early signs non-specific & mild
  - Respiratory signs often seen at end of disease process
    - Weakness
    - Exercise intolerance
    - Dyspnea with open mouthed & abdominal breathing
    - Loss of body condition
    - Change in voice quality or loss of voice
    - Pastel green mutes
Diseases with a similar presentation

- DDx for a falcon with weight loss, laboured breathing & a raised white cell count:
  - Chlamydiosis
  - Bacterial lower respiratory tract disease
  - Inhaled FB
  - Mycobacteriosi
  - Neoplastic disease
Aspergillosis Diagnosis

- Because of vague clinical signs in the early stages, early diagnosis is difficult
  - Clinical examination
  - Blood test - haematology
  - Radiography
  - Endoscopy
  - Cytology of biopsies
  - Culture of biopsies/tracheal swabs
Aspergillosis Diagnosis

- Radiography
  - Hyperinflation of abdominal airsacs
  - Focal densities in lungs/airsacs
  - Reduced coelomic cavity detail
  - Prominent bronchial pattern
  - Asymmetry of the air sacs because of air sac consolidation or hyperinflation
  - Visible caudal airsac line
Full Check Doctor

- Falconer brings falcon from dealer or local breeder for pre-purchase exam

- No history/signs – birds are being sold as ‘healthy’

- ? Pre-clinical aspergillosis

- Aspergillosis - main reason for rejection

- A diagnosis ASAP - Tomorrow is too late
The need for speed - pre-purchase examinations

- Peculiarity of Arabic falconers
- Traditional
- Accepting of invasive veterinary procedures
Aspergillosis Diagnosis

- **Endoscopy**
  - Routine approach into caudal thoracic airsac & trachea
  - Looking for changes to the lower respiratory tract
  - Other sites e.g. clavicular airsac
Figure 6.3A The respiratory system of the professional bird.

- **Glottis**
- **Larynx**
- **Trachea**
- **Cervical Air Sac**
- **Interclavicular Air Sac**
- **Syrinx**
- **Bronchus**
- **Humeral Air Sac**
  - **Anterior**
  - **Thoracic Air Sac**
    - **Posterior**
    - **Thoracic Air Sac**
- **Mesobronchus**
- **Lung**
- **Abdominal Air Sac**
Aspergillosis Diagnosis

- Vague signs in early stages
  - early diagnosis is difficult

- Clinical examination
- Blood test - haematology
- Radiography
- Endoscopy
- Cytology of biopsies
- Culture of biopsies/tracheal swabs
- Histopathology of biopsies
- Serology, Electrophoresis,
What is really important in the aspergillosis early-diagnosis factory

- Haematology
- Radiography
- **Endoscopy**
- Cytology of biopsies
- Culture of biopsies/tracheal swabs
- Serology, Electrophoresis,
Serum protein electrophoresis (SPE) has gained importance in bird medicine during the last decades (Werner & Reiss, 1986) and it has become evident that interpretation patterns and values vary between different species (Say & Lin, 1998). Identification of the protein fractions in the electropherogram helps in differentiating different techniques. The aim of this study was to evaluate high-resolution agarose gel fluorometry for protein species commonly used in falcons in the Middle East, including avian electrophoresis (Cocks & Bargeton, 1981) and glycine electrophoresis (Foster et al., 1973). Serum protein electrophoresis values were established for clinically healthy falcons. To determine the usefulness of SPE as an accessory tool to indicate pathologic conditions, serum samples from falcons with confirmed Aspergillus sp. infections were then analyzed and compared with values from clinically healthy birds.

Materials and methods

Material.

Samples were collected from the Dubai Falcon Hospital from 2002-2005. Healthy falcon: Samples from 57 clinically healthy falcons and 30 falcons with aspergillosis were analyzed. Infections with Aspergillus sp. had been diagnosed endoscopically.

Methods.

Electrophoresis was performed in a SAS 1 unit (Hirer, Sainte La Forest, France) on a high-resolution agarose gel (SAS-SP 24 GB) (Hirer) with two applications at an electric voltage of 100 V for 18 minutes of the electropherogram. Based on the calculation of total protein (TP) and albumin fraction in the electropherogram, pre-albumin, albumin, alpha-globulin, beta-globulin, and gamma-globulin as a comparison for the best overall peak was found by comparing the serum with plasma samples in the last albumin peak most strongly justified due to the Bradford (1976).

Results.

Values for clinically healthy birds are presented in Table 1. There were no statistically significant differences between species (ANOVA; p < 0.05). Albumin values varied significantly lower prediabetes and albumin/albumin ratios and increased alpha-globulin.

Assessment of a commercial sandwich ELISA in the diagnosis of aspergillosis in falcons


A commercial sandwich assay (Molecular Aspergillus test, Bio-Rad) developed for the detection of galactomannan, a major cell wall constituent of Aspergillus species, was tested for its efficacy in the diagnosis of aspergillosis in falcons. Ninety serum samples from 30 aspergillosis-positive falcons and 120 samples from 142 aspergillosis-negative falcons were analyzed. The sensitivity of the test was only 12% per cut-off and its specificity was 100% per cut-off. The test was therefore unsuitable for detecting galactomannan in the serum samples and cannot be used as a screening test for aspergillosis in falcons.

Materials and methods


tables

A commercial sandwich assay (Molecular Aspergillus test, Bio-Rad) developed for the detection of galactomannan, a major cell wall constituent of Aspergillus species, was tested for its efficacy in the diagnosis of aspergillosis in falcons. Ninety serum samples from 30 aspergillosis-positive falcons and 120 samples from 142 aspergillosis-negative falcons were analyzed. The sensitivity of the test was only 12% per cut-off and its specificity was 100% per cut-off. The test was therefore unsuitable for detecting galactomannan in the serum samples and cannot be used as a screening test for aspergillosis in falcons.
Endoscopy images of early aspergillosis
Aspergillosis Diagnosis – Air Sac Cytology

**Early**
- Multinucleated giant cells
- Septated *Aspergillus* hyphae

**Late**
- *Aspergillus* conidiospores, conidiophores & hyphae
- *Aspergillus* spores & macrophages.
Treatment goals for aspergillosis are:

1) Removal of lesions that restrict airflow
2) Killing & elimination of the fungus
3) Provision of supportive care
4) Correct factors associated with cause of disease
Factors Associated with Susceptibility to Aspergillosis

- Immunosuppression
- Pre-existing disease conditions
- Treatment with immunosuppressive drugs
- Exposure to respiratory irritants
- Environmental factors (temperature, humidity)
- Malnutrition
- Other causes of physiological stress
- Falconry training

Correct any contributing factors, in addition to embarking on medical & surgical intervention
Aspergillosis Therapy
Voriconazole

- Excellent activity against *Aspergillus*

- Vfend, Pfizer approved in humans to tx invasive aspergillosis, *Fusarium* and *Scedosporium* infections & resistant candidiasis

- Humans - approved by FDA for:
  - fungal infections where there is a probability of resistance to other available therapies
  - patients who cannot tolerate other therapies
Voriconazole Trial at DFH

- 20 falcons
  - grade 1 - 5 birds
  - grade 2 - 8 birds
  - grade 3 - 7 birds
- Tx oral VCZ + VCZ nebulisation
- 12.5mg/kg bid oral
- Tx range – 18-100 days
  - Complete clinical resolution – 14 (70%) cases
  - Partial resolution – 5 (25%) cases
  - Failure (death) in 1 (5%) case

Aspergillosis Grades

- **Group 1 (early)** - a few endoscopic lesions < 5 mm diameter, cytology consistent with a mycotic infection, no detectable clinical signs.

- **Group 2 (intermediate)** - multiple granulomas and plaques 5-10 mm of diameter, hematology showing leukocytosis and heterophilia, minimal radiographic opacities, culture and cytology positive for *Aspergillus* spp.

- **Group 3 (advanced)** - large and coalescent granulomas (>10mm), marked leukocytosis and heterophilia, general clinical signs of malaise, easily detectable opacities in air sacs and/or lungs, culture and cytology positive for *Aspergillus* spp.
Follow-up of Aspergillosis Cases

Before Tx

After 3mo tx

End tx 5 mo
ID 765: Gyrfalcon young Female III stage

Oral VCZ Endoscopical debulking

Therapy duration 39 days

Complete response

Follow up: normal
Therapy – What We Did/Do

- Combination therapy using voriconazole
  - Oral Voriconazole
  - Nebulisation with voriconazole, terbinifine, amp B or F10.
Posaconazole

- Latest human antifungal agent to be approved
- Excellent in-vitro activity against aspergillosis
- 12.5mg/kg bid used in falcons (Di Somma)
Posaconazole (PCZ)

- results of first use of PCZ in falcons at DFH by Di Somma
- 13 falcons (gyr or gyr hybrids) with confirmed aspergillosis
  - 10 non-responsive to voriconazole
  - 3 cases – only posaconazole used
- 3 falcons – no follow-up
- 2 falcons – anorexia – taken off PCZ
- 6/8 remaining falcons healed after 23-95 days therapy
- Main side-effect noted – anorexia
- Resolved by stopping PCZ for 3-5 days and then putting back on PCZ once a day

- Posaconazole – possible treatment option for valuable cases that are not-responding to Vfend
Figure 1. *Aspergillus* sp. isolated from falcons

Figure 3. E-test showing MIC of *A. fumigatus*
Recommendations to prevent aspergillosis

- Reduce stress (especially newly captured birds)
- Prophylactic antifungals for birds undergoing a change in management (pre-export, hospitalisation), esp high risk species
- Increase ventilation within facility
- High standards of hygiene to maintain a healthy environment (reduce exposure to mouldy vegetation in litter and feed)
- Spray/fog litter/facilities with appropriate disinfectant
Cost of therapy (2008)

- Vfend 50 mg 28 tablets : GBP 299

To treat a 1 kg bird

- Cost 12.5 mg : GBP 2.6

- Cost/30 days treatment GBP 150
Therapy

- **Surgery**
  - Remove granulomas from airsacs or trachea or syrinx
  - Direct application of antifungal agents to the lesions (endoscopy)

- **Other therapy**
  - Supportive therapy -
  - Avoid steroids, cortisone-treated rabbits susceptible to invasive aspergillosis

- **Immune stimulation**
  - Interferon $\gamma$ & colony stimulating factor trials promising in humans
Duration & Efficacy of Therapy

- **How long is treatment necessary**
  - Treatment in humans often extended for 6-12 months
  - Veterinary recommendations vary from 2 to 6 months

- **Are recovered animals susceptible to rechallenge with aspergillosis?**
  - Convalescence from aspergillosis in turkeys did not confer protection against rechallenge, instead decreased resistance to subsequent infection
  - Recrudescence of infections in falcons that have recovered from aspergillosis is common

- **Vaccination?**
  - No vaccine commercially available for any veterinary species
Complications of Fungal Infections & Antifungal Therapy
Thanks

- Antonio Di Somma for sharing his data on posaconazole
- Chris Johnson for some images