Aspergillosis: An Overview

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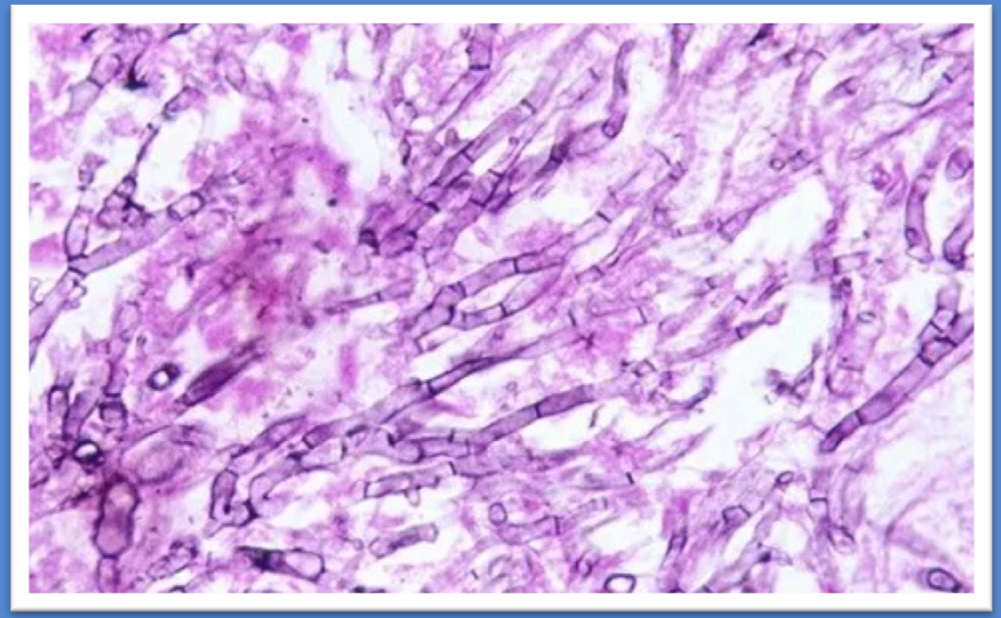
Aspergillosis

- Most common fungal disease in birds
- Caused most commonly by *Aspergillus fumagatis*
  - Ubiquitous, saprophytic fungus
  - Spores are conidia, branching filaments are hyphae
  - Hyphae are septate and branch at 45° angle
- Other *Aspergillus* species less common
  - *A. flavus*
  - *A. niger*
  - *A. glaucus*
  - *A. nidulans*
  - Others
- May cause acute or chronic infection
- Typically is an individual infection; less commonly epizootic



Dichotomously branched, septate hyphae of Aspergillus fumigatus in lung tissue section after Methenamine silver stain

From https://microbeonline.com

Risk of Infection

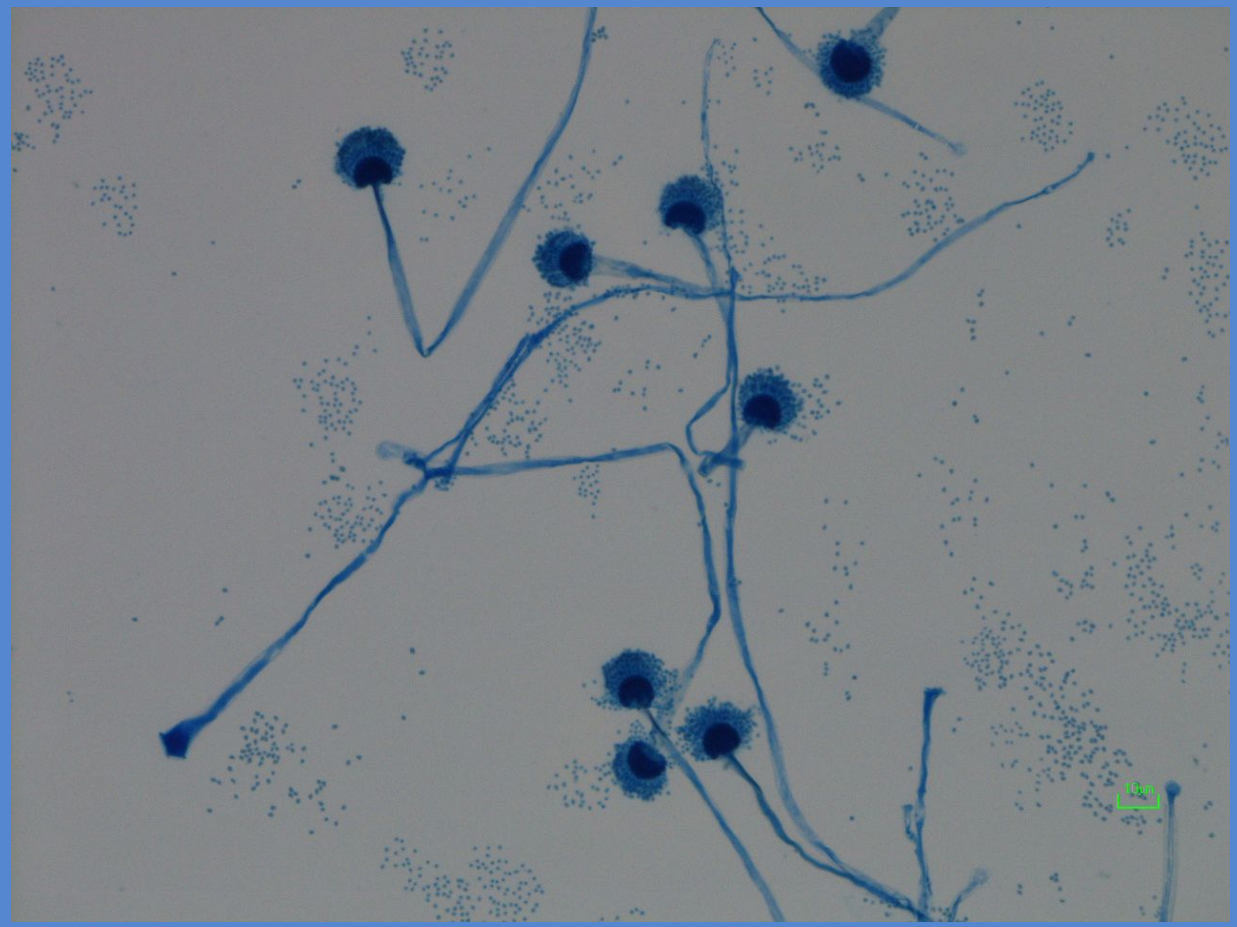
- All bird species are susceptible
  - Some are predisposed: birds of prey, African gray parrots, blue-fronted Amazons, pionus, seabirds
- Most commonly contracted via inhalation or ingested in moldy food
- Increased risk with:
  - Exposure to excessive number of spores
  - Poor ventilation
  - Poor sanitation/overcrowding
  - Humid environment
  - Immunosuppression from other diseases, stress, reproductive activity, wild birds in captivity

Pathogenesis

- Spores are most commonly inhaled
- Less often, infection occurs through skin and eye
- Spores may be trapped in nasal cavity, trachea/syrinx, lungs, air sacs
- Spores are attacked by phagocytic epithelial cells, macrophages
- If an overwhelming number of spores are present, they germinate into hyphae
- Hyphae invade tissue, causing plaques and necrosis that obstruct trachea, bronchi, air sacs → tissue infiltration & granulomas
- Organs and tissues adjacent to respiratory tract can be infected
- Hematogenous spread of infection also occurs

Clinical Signs

- Depend on:
  - Degree of spore exposure
  - Route of infection
  - Presence of pre-existing disease
  - Immune response of host
- May be localized or diffuse
- Generally, there are 2 forms:
  - Acute
  - Chronic



Clinical Signs

- Acute form:
  - From exposure to an overwhelming number of spores
  - Rapid onset of signs:
    - Dyspnea
    - Anorexia
    - Tail bobbing
    - Vomiting
    - Crop stasis
    - Ascites
    - Polyuria/polydipsia
    - Cyanosis
    - Death within a week
  - Necropsy - congested air sac & lungs with mucoid exudate and small nodules; large granulomas are absent

Clinical Signs

- Chronic form:
  - Generally associated with underlying immunosuppression
  - May be localized or disseminated disease
  - Clinical signs:
    - Decreased appetite
    - Weight loss
    - Lethargy
    - Loss of or change in voice
    - Cough
    - Vomiting
    - Polyuria/polydipsia
    - Gradual onset of dyspnea/cough
  - Necropsy - granulomas are found throughout respiratory tract; airsacculitis

Clinical Signs

- Chronic form – may be localized or disseminated
  - Localized disease
    - Rhinitis/sinusitis
      - Nasal discharge, rhinolith formation, malformed beak/cere/nares
    - Keratitis
      - Blepharospasm, photophobia, periorbital swelling, swollen eyelids, cloudy cornea, yellow ocular discharge
  - Disseminated disease
    - Encephalitis/meningoencephalitis
      - Weakness, paralysis, unilateral wing droop, ataxia, torticollis, tremors

Diagnosis

- Often very difficult
- There is no single *definitive* test for diagnosis
- Based on accumulation of evidence from history, signs, test results
- Diagnosis is frequently made at necropsy:
  - Nodules in lungs
  - Plaques in air sacs
  - Cytology/histopathology/culture of fungus

Diagnosis

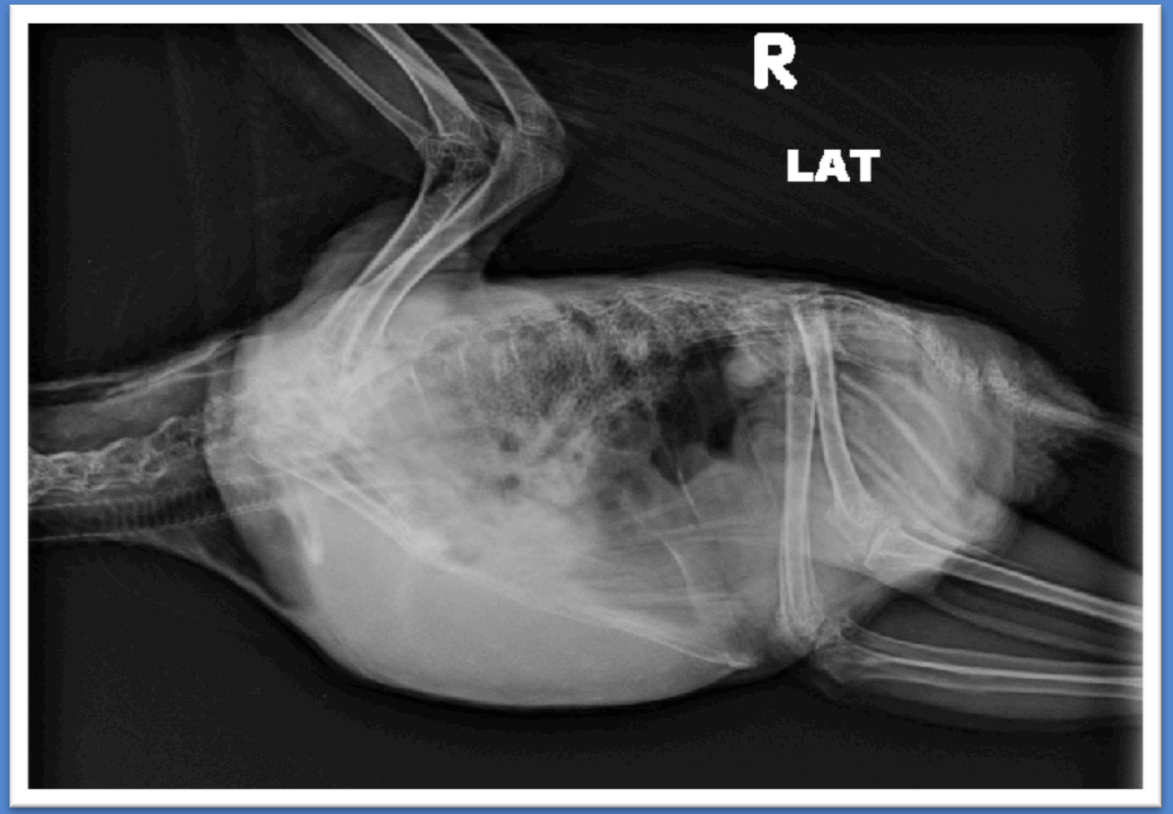
- Hematologic findings:
  - Leukocytosis: 20,000-100,000+ wbc/µl (normal wbc count if immunosuppressed)
  - Heterophilia
  - Left shift
  - Monocytosis
  - Lymphopenia
  - Non-regenerative anemia
- Serum chemistry findings:
  - Elevated total protein concentration (hypoproteinemia if immunosuppressed)
  - Increased β-globulins (acute), γ-globulins (chronic)
  - Decreased albumin concentration

Diagnosis

- Serologic findings:
  - Can't be relied on because:
    - Antibodies to *A. fumigatus* do not form until 10-14 days after antigen exposure
    - Immunosuppressed birds may *not* produce antibodies
    - Healthy birds may have high antibody titers from exposure
  - Indirect ELISA
    - Detects antibodies against *Aspergillus* spp.
    - Especially helpful in diagnosing subclinical infection
    - Can be false-negative
    - May be more reliable in Falconiformes and less so in other orders
  - Direct ELISA
    - Detects fungal antigen galactomannan (in fungal cell wall)
    - Commonly elevated in psittacine birds with aspergillosis
    - May be false-negative

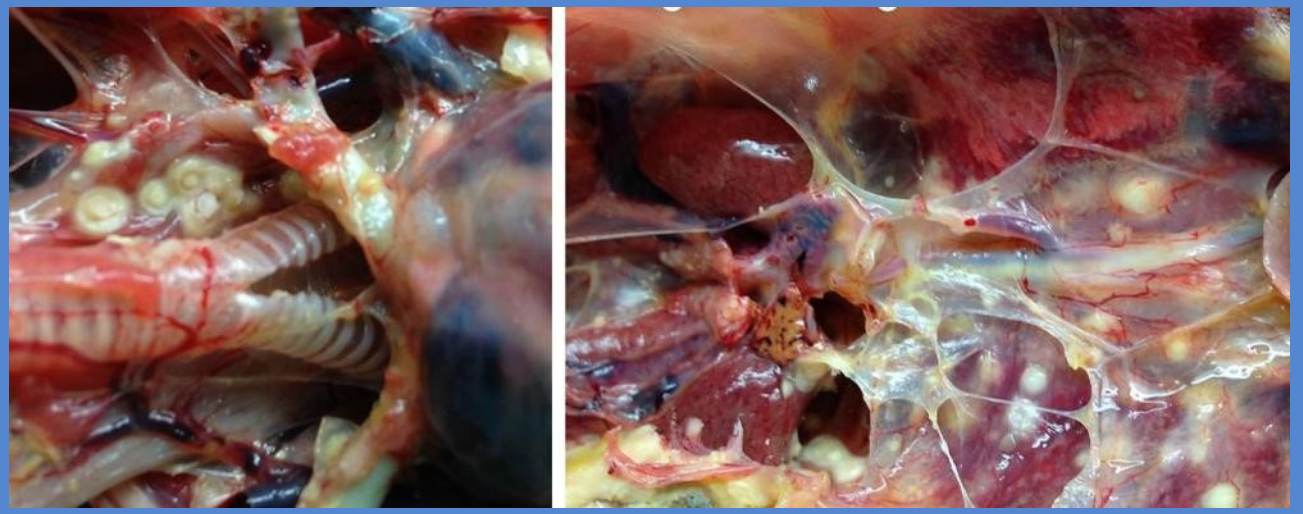
Diagnosis

- Radiographic findings
  - Changes are often detected only late in disease
    - Bronchopneumonia/prominent parabronchial pattern
    - Thickened air sac walls/airsacculitis
    - Distinct nodular lesions
    - Air sac hyperinflation from airway obstruction/rigid air sacs
    - Organ enlargement with systemic infection
- CT/MRI
  - Especially useful to detect small lesions not visible on radiographs



Diagnosis

- Endoscopic findings:
  - Endoscopy is useful
    - To evaluate choana, trachea, syrinx, lung, air sacs, coelomic cavity
    - To obtain diagnostic samples:
      - Cytology
      - Histopathology
      - Culture
        - Gold standard
        - Positive culture not diagnostic because *Aspergillus* fungus is ubiquitous/contaminant
        - Negative culture doesn't rule-out infection
    - To follow treatment progress



Adapted from MerckVetManual.com

Treatment

- Difficult because of:
  - Limited knowledge of antifungal drug pharmacokinetics in different species
  - Difficulty in getting drugs to penetrate granulomatous tissue
  - Presence of concurrent disease/immunosuppression
  - Need for long term treatment (typically ≥ 4-6 months)
- Generally involves:
  - Debulking/surgical removal of granulomas, if possible
  - Long term systemic antifungal therapy – oral or intravenous
  - Topical therapy:
    - Nebulization
    - Nasal/air sac flushing
    - Endoscopic or surgical irrigation of lesions

Adapted from Wellehan JFX, Lierz M, Phalen D, et al: Infectious Disease. In Speer B, editor: *Current Therapy in Avian Medicine and Surgery,* St. Louis, Missouri, 2016, Elsevier, pp 22-106

Antifungal Agents Used for Treatment of Avian Aspergillosis

Antifungal Agent	Administration Route	Dose
Amphoteracin B	Intravenous	1.5 mg/kg q8h 3-7 days (most species)
	Intratracheal/nasal flush	1 mg/kg q8-12h, dilute to 1 mL with sterile water (psittacines, raptors)
	Nebulized	7 mg/mL 15 min q12h (most species)
Clotrimazole	Nasal flush	1% solution
	Nebulized	1% solution, 30-60 min
Enilconazole	Nebulized	0.1 mL/kg in 5 mL sterile water, 30 min q24h 5 days on/2 days off (raptors)
Itraconazole	Oral	5-10 mg/kg q 12-24h (toxicity is reported in Gray parrots so lower does to 2.5-5 mg/kg PO q24h)
Ketoconazole	Oral	10-30 mg/kg q12h 21 days
Terbinafine	Oral	15-30 mg/kg q12h
	Nebulized	1 mg/mL solution (can be combined with itraconazole)
Voriconazole	Oral	10 mg/kg q 12h (chickens, pigeons, Gray parrots); 12-18 mg/kg q12h (Gray parrots); 12.5 mg/kg q12h (falcons)

Treatment

- Polyene drugs
  - Amphotericin
    - Macrolide that binds to ergosterol in fungal cell membrane, increasing membrane permeability, killing cell
    - Nephrotoxic in mammals but not in birds
    - Need to monitor avian renal function with this drug, regardless
    - Given intratracheally, intravenously, nebulized, in sinus flush
    - Dilute in water (not saline – inactivates) if use topically because irritating

Treatment

- Azoles
  - Inhibit cytochrome P450 enzymes that form ergosterol in fungal cell membrane and interfere with fungal structure and growth
  - Are fungistatic at doses typically used in birds
  - Need several days to reach steady state concentrations & months to achieve therapeutic effects
  - *A. fumigatus* infecting birds is becoming increasingly resistant to azoles

Treatment

- Azoles
  - Itraconazole
    - Commonly used to treat aspergillosis
    - Administered orally
    - Therapeutic dose may vary by species
    - African gray parrots may develop anorexia, depression, death at doses used in other species, so use at lower dose

Treatment

- Azoles
  - Voriconazole
    - Better tissue distribution (to respiratory tract and brain) than itraconazole
    - Well-tolerated by African gray parrots
    - Administered orally, intravenously, and topically
    - Topical treatment preferred over systemic therapy to minimize adverse effects when treating localized infections in lungs, skin, upper respiratory tract (nose, sinuses)
    - Doesn't achieve therapeutic plasma or lung concentrations when nebulized
    - May be liver-toxic, so must monitor hepatic enzymes during treatment

Treatment

- Allylamines
  - Terbinafine
    - Inhibits squalene epoxidase enzyme in ergosterol synthesis, upsetting fungal membrane
    - Fungicidal
    - Administered orally (10-15 mg/kg PO q 12-24h) or nebulized
    - Pharmacokinetic studies in African gray parrots and raptors showed no therapeutic concentrations achieved at doses usually used to treat birds
    - Anecdotal reports in birds show successful treatment +/- itraconazole, especially as an alternative treatment, where conventional therapies have been ineffective

Prevention

- Minimize risk factors
  - Reduce exposure to fungal spores (dust)
  - Treat birds at high risk (i.e. captive raptors) prophylactically
  - Nebulize antifungal disinfectants to reduce environmental contamination in commercial facilities containing live animals where risk of infection is high

Case Report #1

- 5-month-old unknown sex Sulfur-crested cockatoo purchased at pet store
- Presentation: 3-day history of bilateral leg paresis; otherwise bright and alert, eating (pelleted diet) well. Owner thought bird flew into wall or was exposed to insecticide
- Physical examination: decreased grasp but positive withdrawal of bilateral legs; normal cloacal and tail tone; no respiratory tract signs
- Blood work: PBFD +
- DDx: aspergilloma with spinal cord involvement vs. trauma vs. lead toxicosis vs. pyrethrin exposure

Case Report #1

- CT scan:
  - Poorly defined, homogenous, non-contrast-enhancing soft tissue density occupies middle third of the right dorsal lung and slightly crosses over midline to medial aspect of left lung
  - Where bone contacts this soft tissue density, right pedicle of T2 is absent, and vertebral bodies of T2-4 are sclerotic

Case Report #1

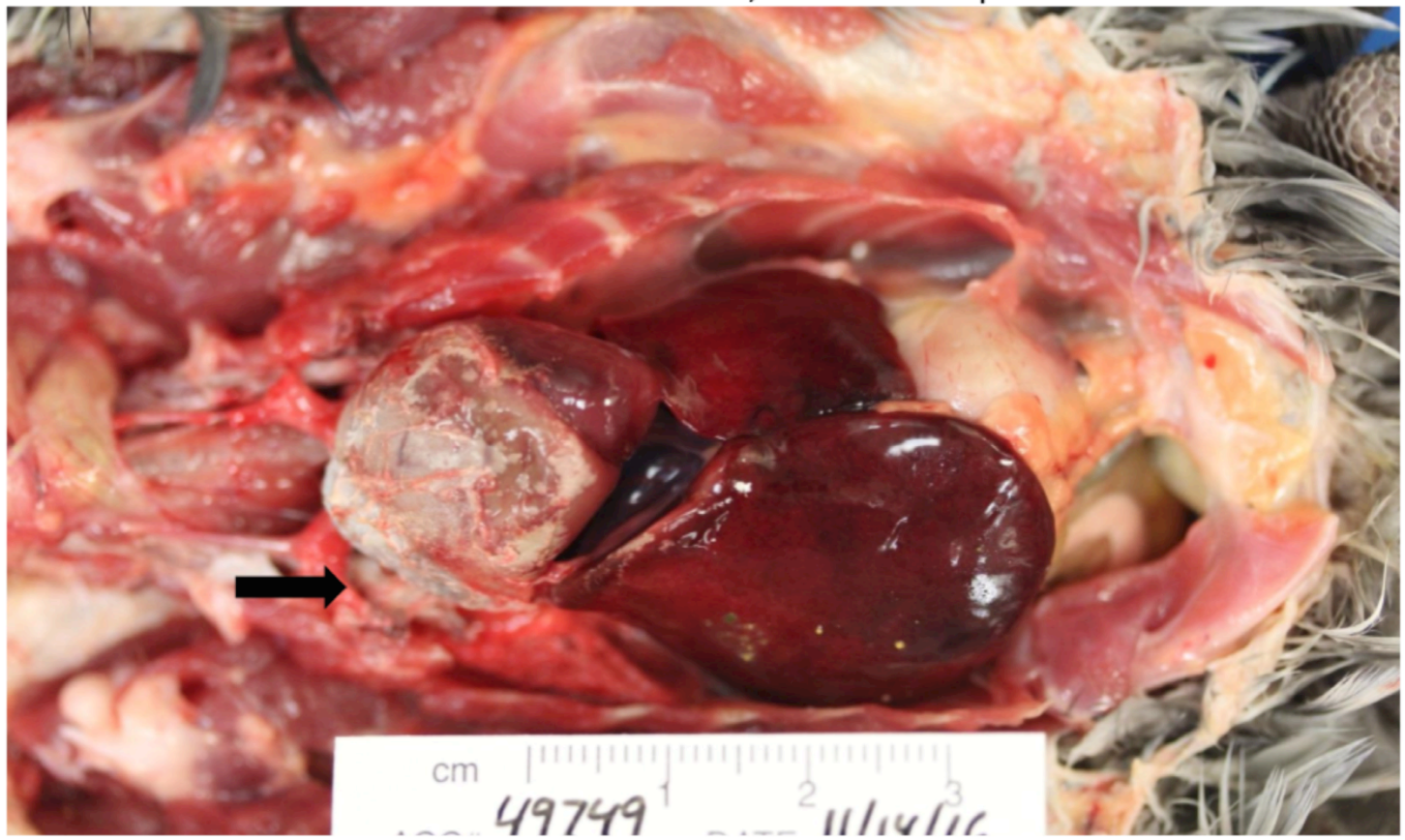
- **Diagnosis:**
  - **An infectious/inflammatory etiology (i.e. aspergillosis) was suspected over neoplasia for the pulmonary and bone lesions, given the imaging characteristics and clinical history**
  - **Neither toxin nor hemorrhage would affect bone this way; osseous changes to vertebral column, especially lysis of the pedicle, could implicate spinal cord involvement by infectious/inflammatory or neoplastic disease, to explain limb paresis**

Case Report #1

- Euthanized due to poor prognosis with PBFD
- Post-mortem findings:
  - Gross:
    - A focal, brown to tan, homogenous, firm nodule (1.5 x 1.8 x 1.2 cm) within right lung and marginally within left lung
    - Trachea, syrinx, air sacs are within normal limits (WNL)
    - Spinal cord is within vertebral canal and looks grossly WNL

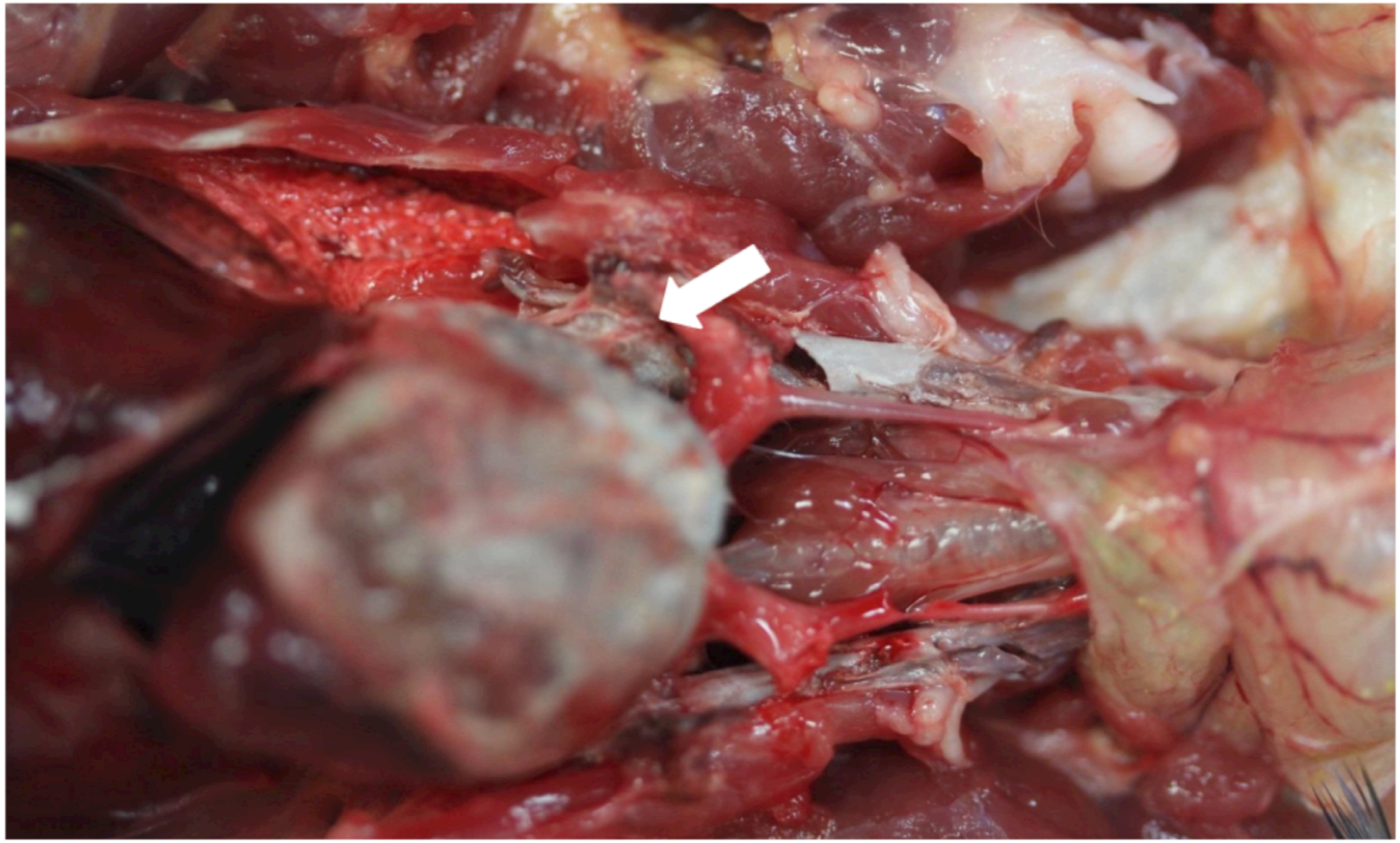
Case Report #1

Viscera in situ: hepatomegaly, granuloma in right lung (arrow), euthanasia artifact over heart, reduced adipose tissue



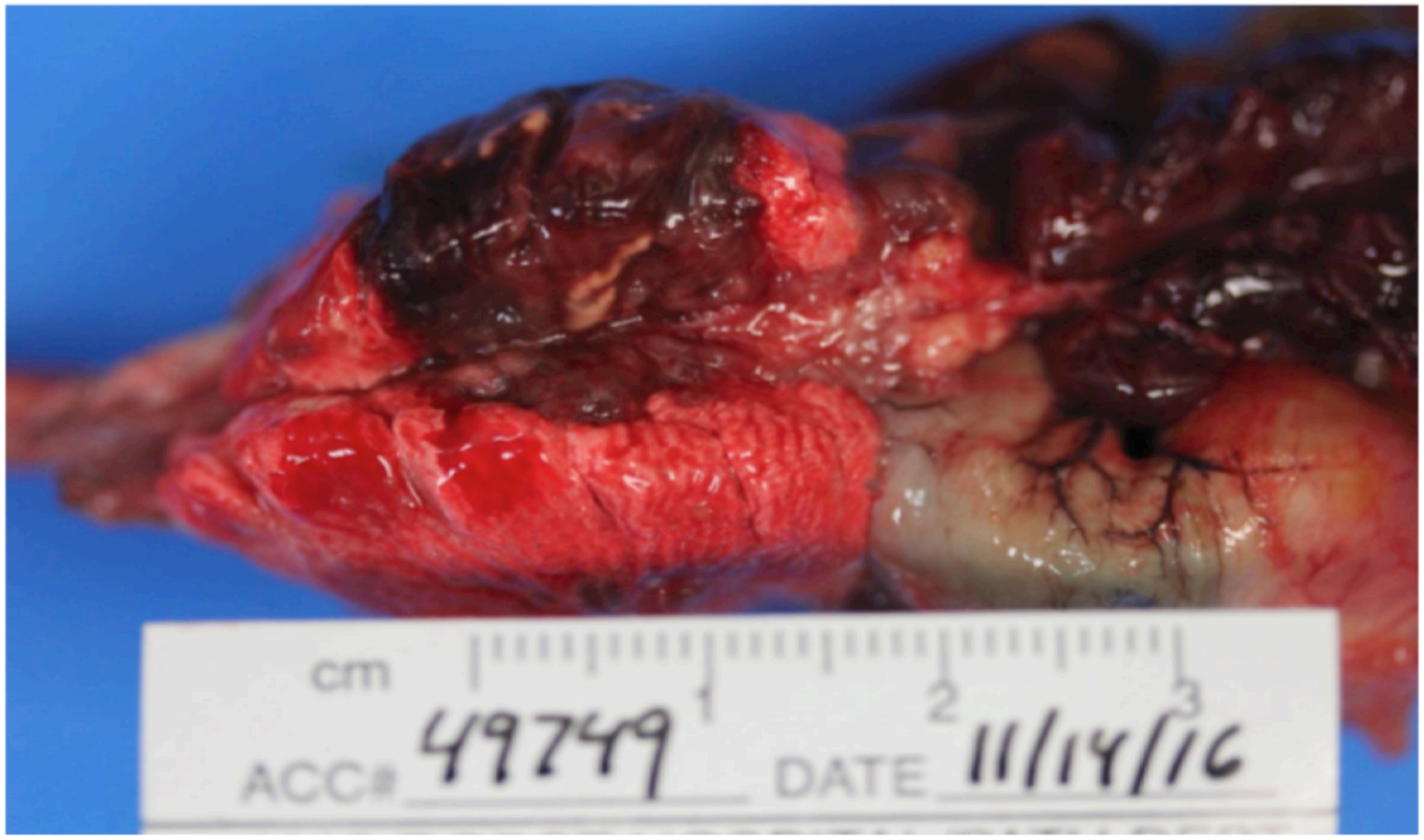
Case Report #1

Right lung granuloma



Case Report #1

Viscera removed, pulmonary granuloma



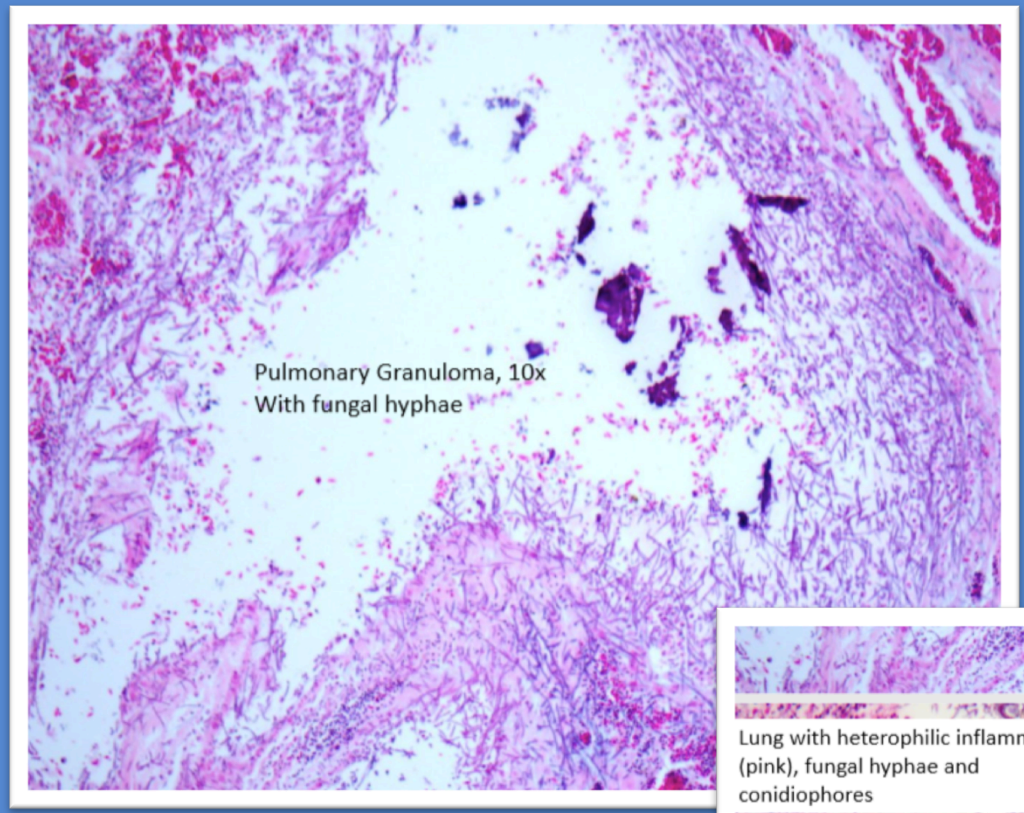
Case Report #1

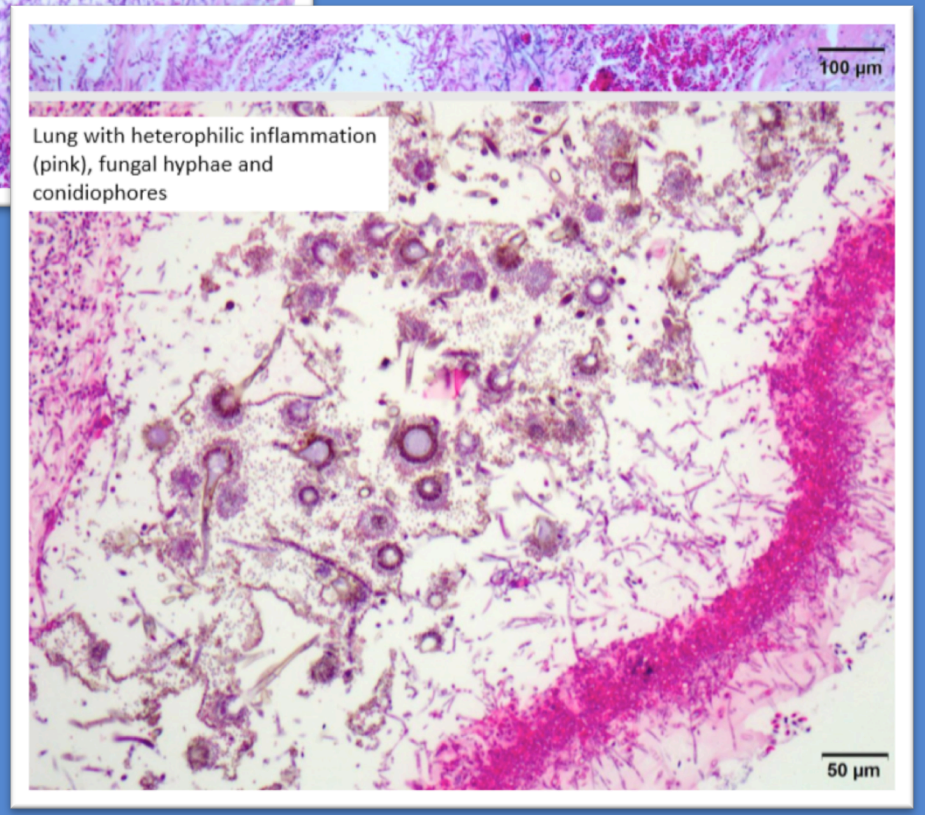
Case Report #1

- Post-mortem findings:

  Histopathology:

  - Lungs: granulomatous heterophilic necrotizing pneumonia, airsacculitis, and fibrinoid arteritis with a myriad of intralesional fungal hyphae and conidiophores (*Aspergillus* spp)
  - Aorta: Arteritis and periarteritis, heterophilic, granulomatous with intralesional fungal hyphae



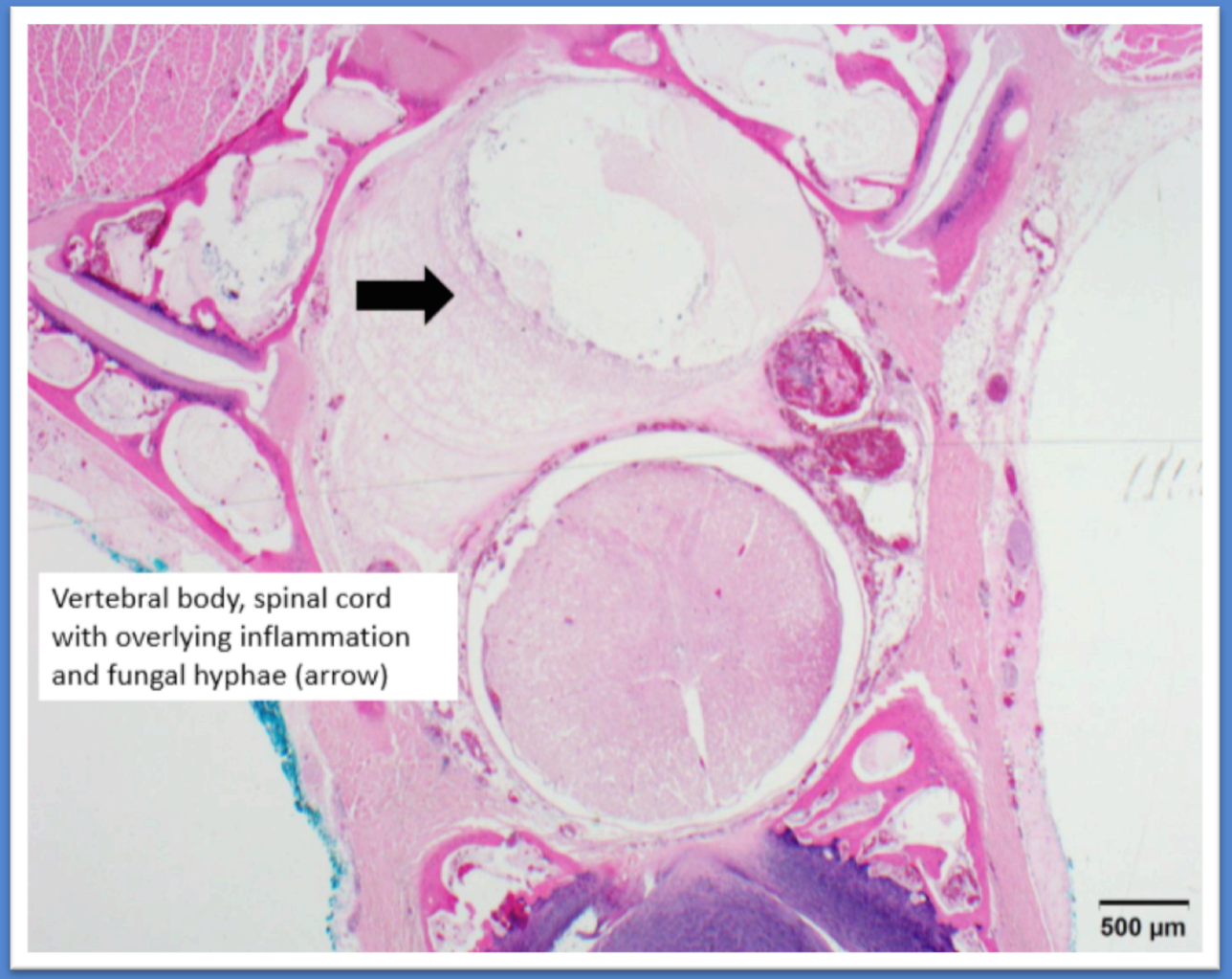


Case Report #1

- **Post-mortem findings:**

  Histopathology:

  - **Vertebrae: myelitis, granulomatous, heterophilic, multifocal with intralesional fungal hyphae; regional fibrin thrombi with intralesional fungal hyphae**

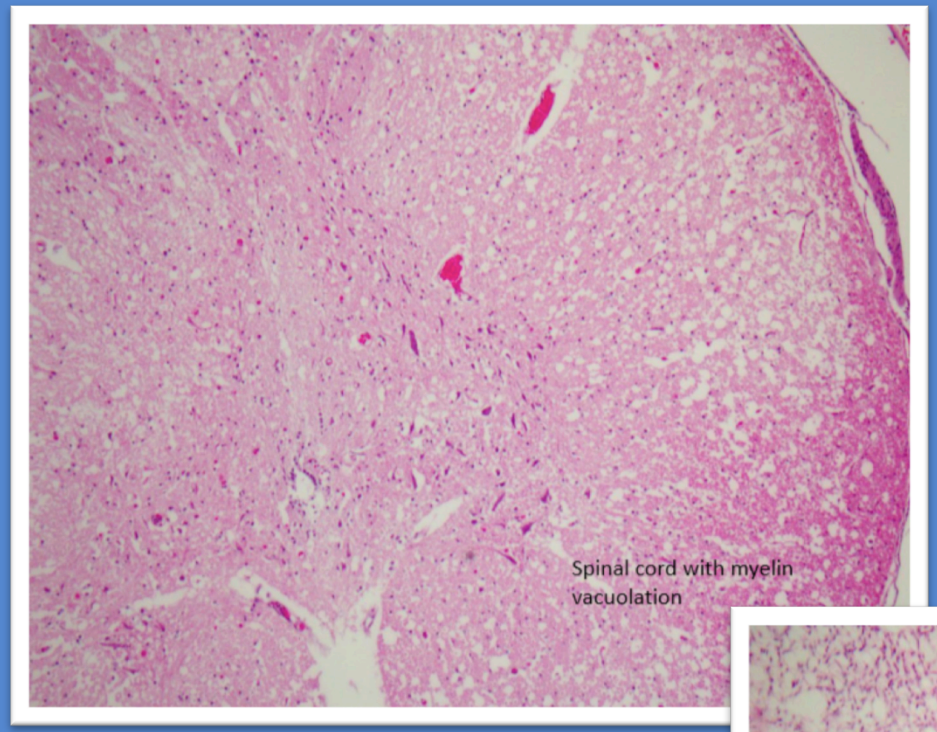


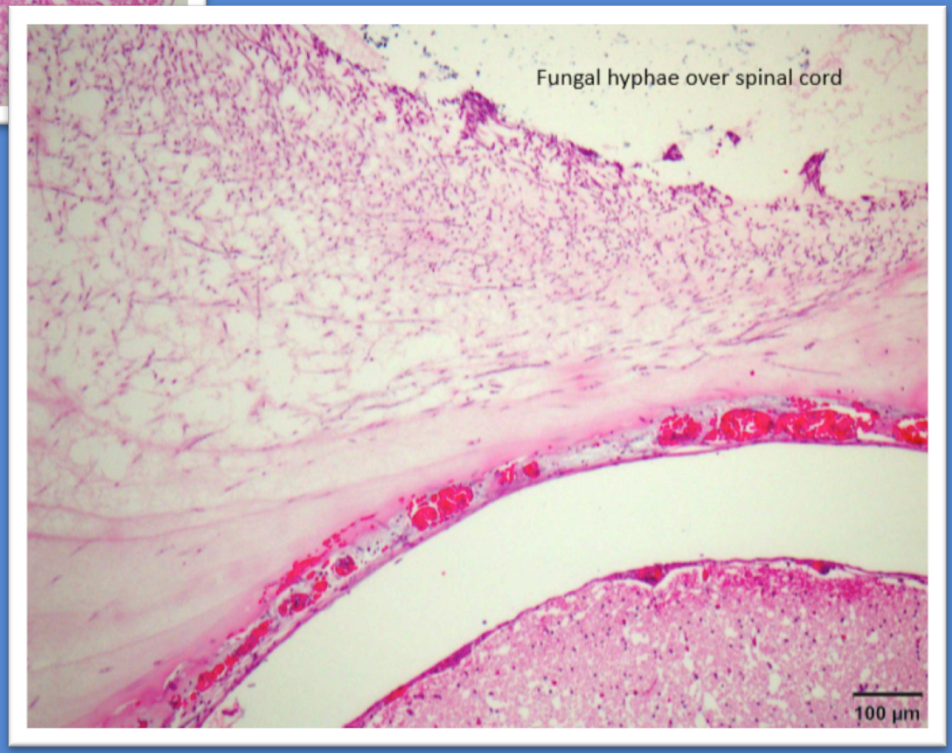
Case Report #1

- **Post-mortem findings:**

  Histopathology:

  - **Spinal cord: vacuolation, multifocal to coalescing and marked, with Gitter cell infiltration, hemorrhage; minimal leptomeningitis with intralesional fungal hyphae**



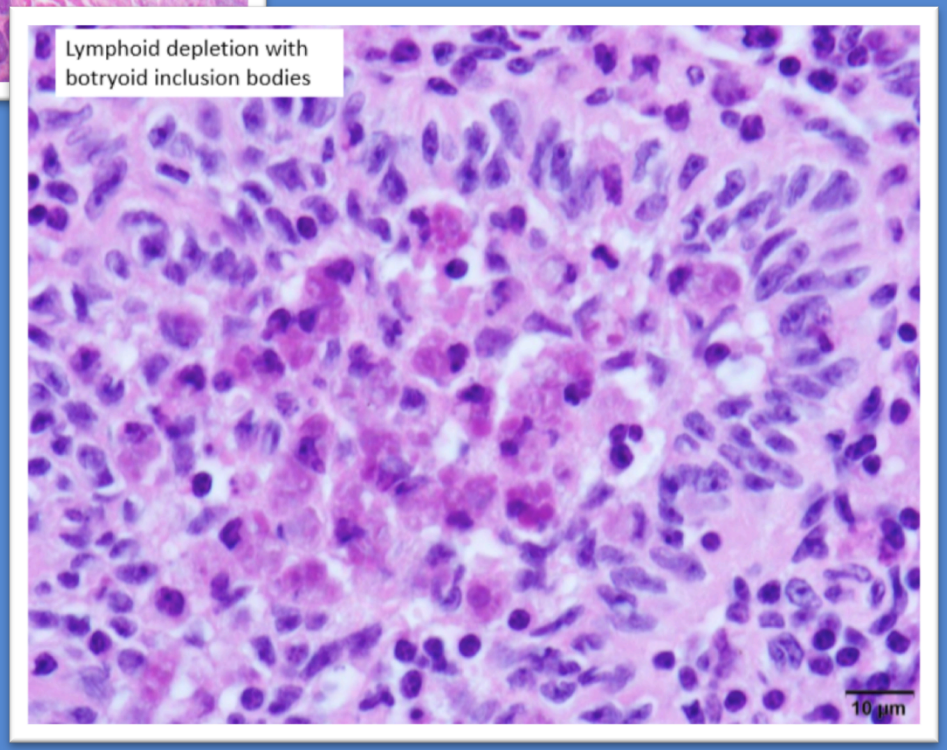


Case Report #1

- **Post-mortem findings:**

  Histopathology:

  - **Bursa: lymphoid depletion with mild/multifocal lymphoplasmacytic bursitis, and intralesional botryoid inclusion bodies (rule-out circovirus infection)**

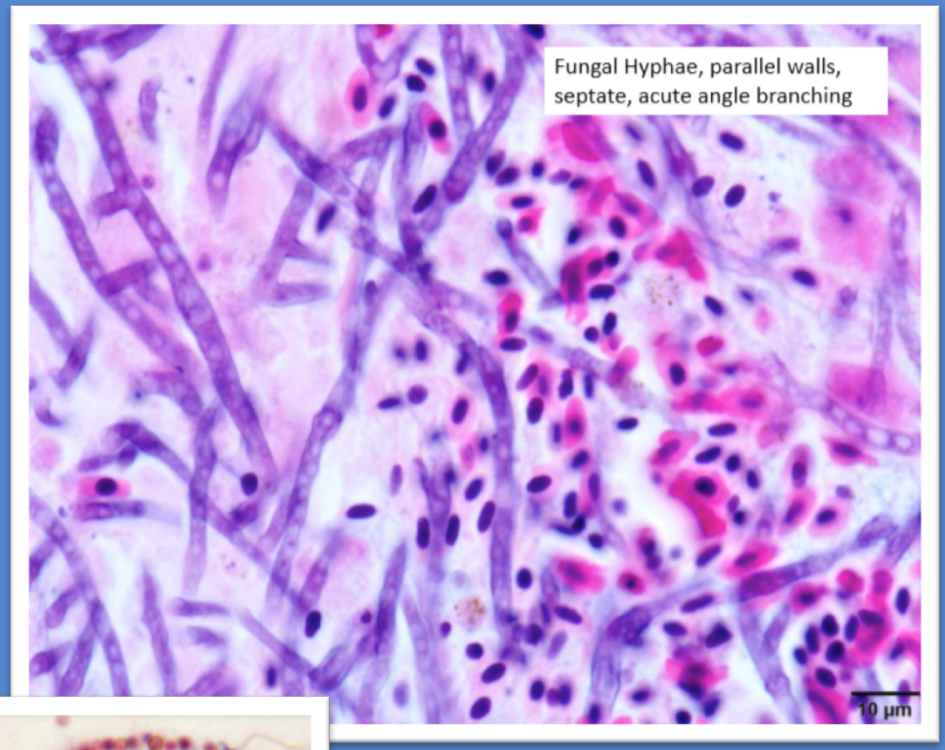


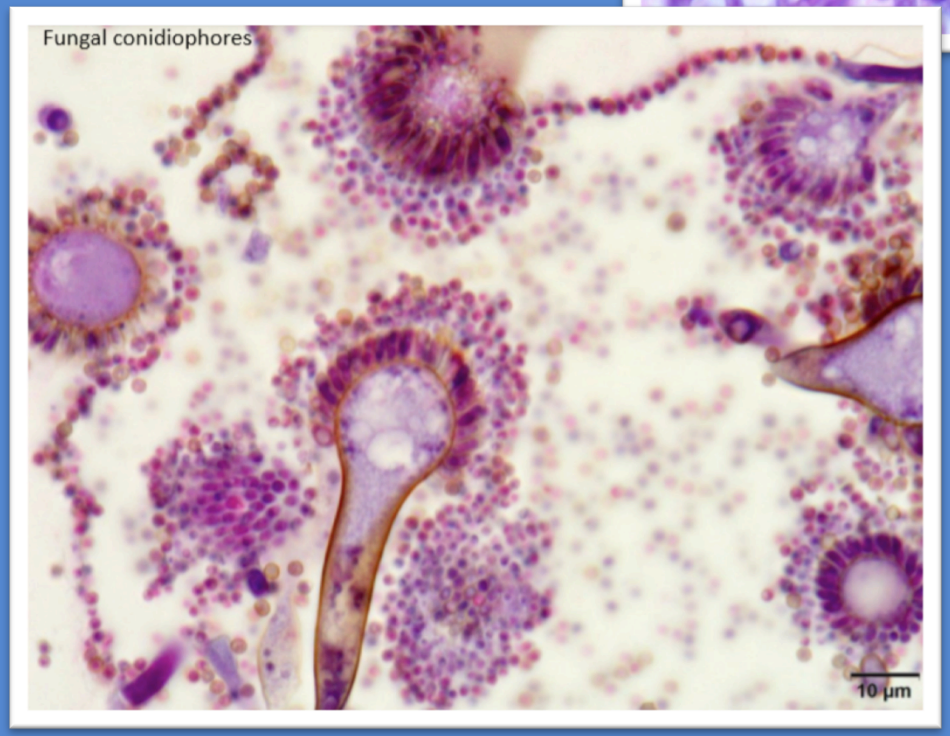
Case Report #1

- **Post-mortem findings:**

  Cytology:

  - **Right lung nodule: scattered inflammatory cells, intermixed with erythrocytes and fungal hyphae. Hyphae have parallel walls, are septate, and exhibit acute angle branching (rule-out aspergillosis)**





Case Report #1

- Pathologist comment:
  - Histologic evaluation confirms presence of necrotizing, fungal pneumonia with inflammation of regional, large caliber arteries
  - Extension of fungal hyphae into adjacent vertebrae was also observed, with inflammatory cells and fungal hyphae within the marrow cavity and leptomeninges
  - Thrombi with intralesional fungi were present within vessels; thus concurrent ischemic damage to spinal cord is an additional possible contributing factor to clinical signs. Although significant inflammation was not observed within the spinal cord parenchyma, it is suspected, and may not have been represented within the samples obtained for histopathology
  - Myelin vacuolation may be secondary to compression, inflammation and/or ischemic damage and explain the clinical neurologic signs

Case Report #1

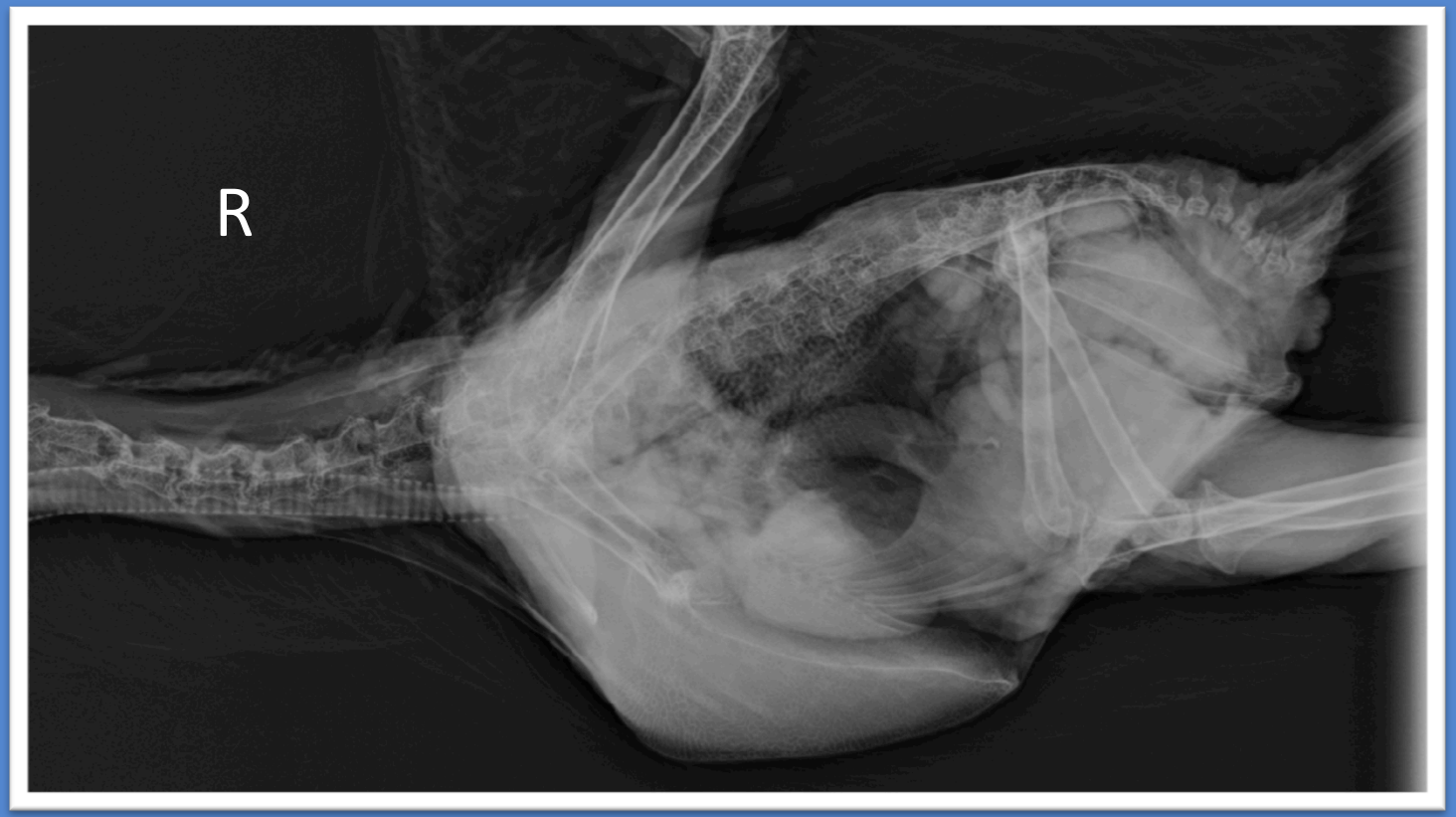
- This case demonstrates an *unusual* presentation of aspergillosis in a psittacine bird: peripheral neuropathy
- Aspergillosis can spread easily from respiratory tract to surrounding organs and bone
- Bird in this case likely was predisposed to infection due to underlying immunosuppression (PBFD+)
- Must keep aspergillosis on DDX list even if respiratory signs not present

Case Report #2

- 10-year-old male Moluccan cockatoo, 0.90 kg
- Presentation: 5-day history of not talking, decreased activity, eating well
- Physical examination: lethargy, moist/hoarse-sounding upper respiratory noise when tries to vocalize; no audible abnormal lower respiratory sounds; clear lungs/air sacs, eupneic
- Blood work: chemistry, CBC/differential, serum protein EPH, *Aspergillus* antibody titer and galactomannan level all WNL

Case Report #2

- Radiographs
  - Clear lungs and air sacs
  - No obvious respiratory tract involvement

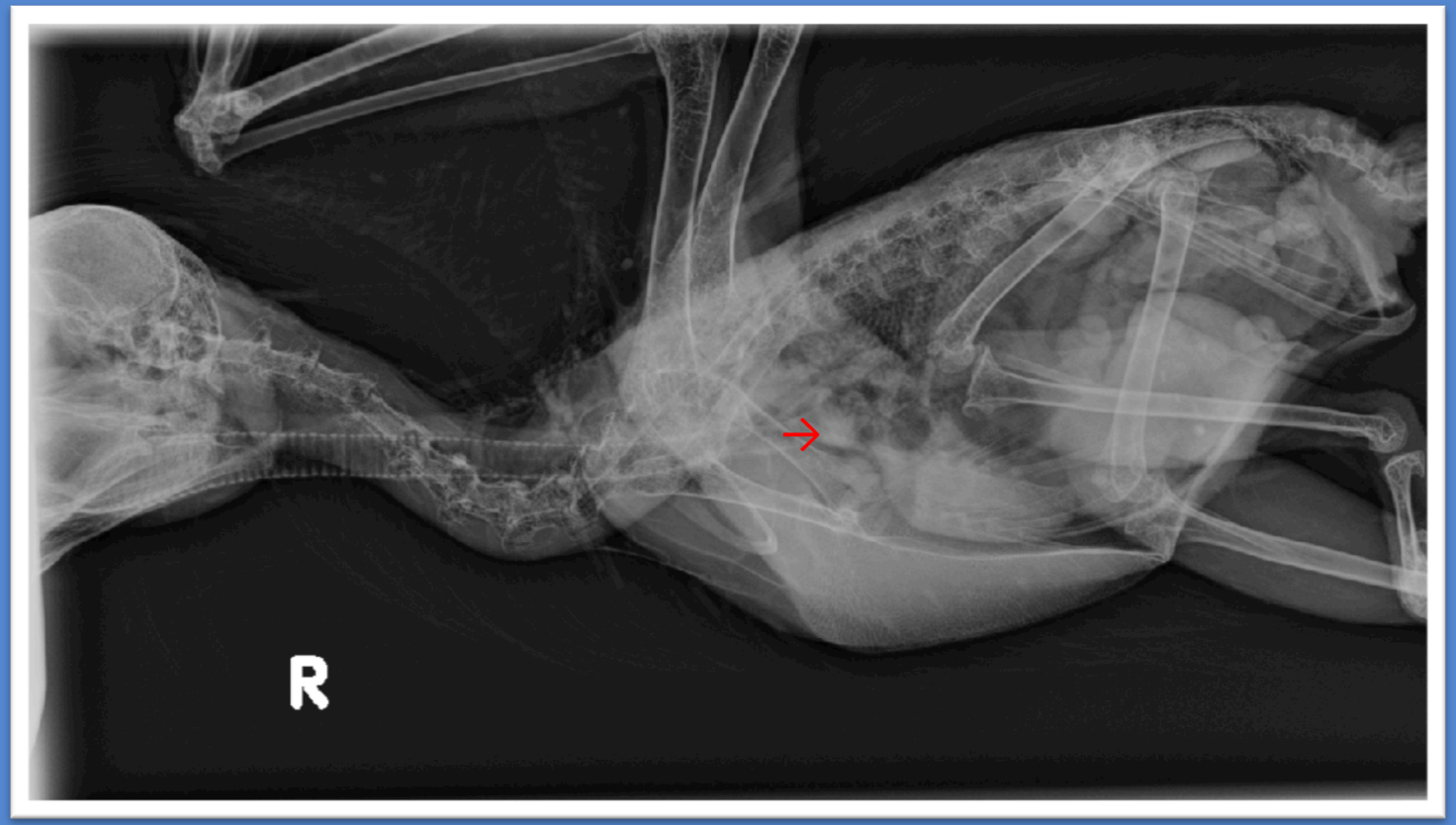


Case Report #2

- Started on Meloxicam 0.5 mg/kg PO q24h, Enrofloxacin 15 mg/kg PO q12h x 14 days
- Audible inspiratory wheeze began within 2 days
- Started Itraconazole 10mg/kg PO q12h; nebulization with Amphoteracin & Piperacillin q12h
- No improvement after 7 days; worsening wheeze
- Rechecked radiographs

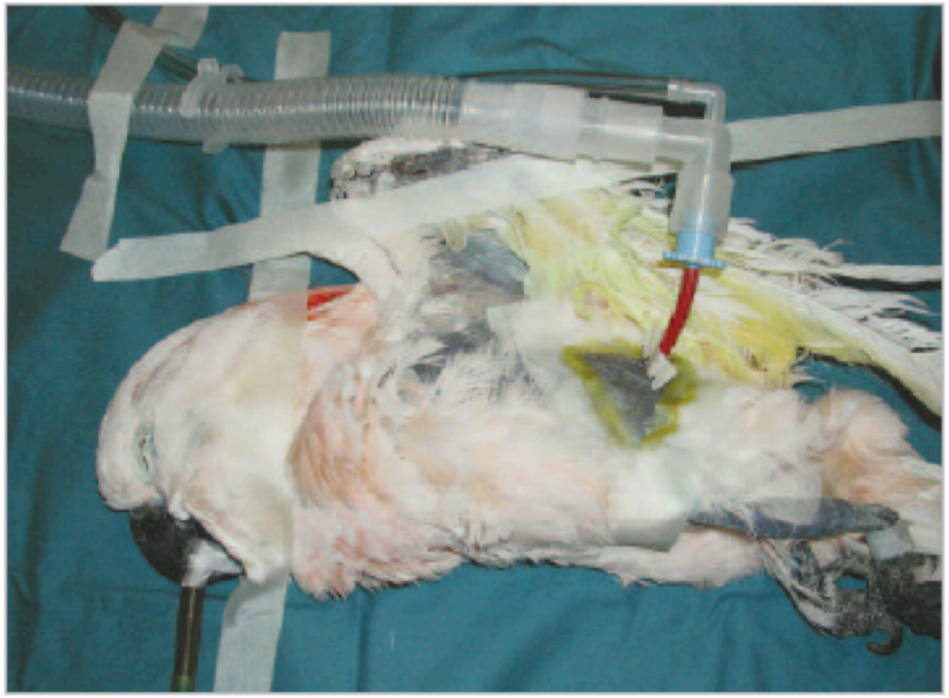
Case Report #2

- Recheck radiographs
  - Clear lungs and air sacs
  - Syringeal opacity present – R/O granuloma?



Case Report #2

- Anesthesia:
  - Butorphanol 1 mg/kg IM
  - Midazolam 1 mg/kg IM
  - Isoflurane 2% via facemask
  - Placed 5 Fr red rubber air sac tube into left caudal thoracic air sac, secured with 3-O nylon and butterfly tape in Chinese finger cuff pattern, & administered isoflurane via air sac tube
  - Maintained under isoflurane anesthesia with PPV via air sac tube



Laurel Degernes

Figure 6. Anesthetized Moluccan cockatoo in lateral recumbency, with a cannula placed in the left caudal thoracic air sac and secured in place with a butterfly tape tab and two sutures. The anesthesia circuit is connected to an adapter on the cannula, and the bird is being maintained under anesthesia using positive pressure ventilation.

Adapted from Vetfolio.com from Dr. Laurel Degernes

Case Report #2

- Tracheoscopy
  - Inserted 2.7 mm rigid endoscope into trachea
  - Visualized large yellow plaque adhered to syrinx, occluding opening to bronchi bilaterally
  - Passed biopsy forceps and grasping forceps alongside scope to break up plaque
  - Submitted samples for histopathology, cytology, aerobic and fungal cultures
  - Repeatedly attempted to suction with 8 Fr red rubber catheter passed alongside scope
  - Very difficult to break up plaque; tracheal walls very inflamed
  - Infused amphotericin 5 mg/ml (0.17 ml) in 5 ml sterile water over syrinx via 5 Fr red rubber tube
  - Administered LRS 50 ml SC
  - Awoke uneventfully

Case Report #2

Endoscopic view:

Adapted from University of Georgia Veterinary School website, https://.vet.uga.edu

Mycotic granuloma occluding the entire distal trachea of a cockatoo. The biopsy forceps are being advanced to biopsy and debride the lesion while anesthesia is maintained via an air sac tube.

Case Report #2

- Bird continued on oral meloxicam, enrofloxacin, itraconazole; nebulized amphotericin & piperacillin
- Results:
  - Cytology:

    - Microscopic Findings: heterophilic and macrophagic inflammation

    - Comment: Sample is composed mostly of chronic-active inflammation and normal appearing epithelial cells.
    - While there is a rare suggested single fungal spore, no definitive determination can be made as to whether there is a true fungal etiology for this granuloma. Obvious bacteria are not identified, but culture is pending. A biopsy of the granuloma, pending, may provide better information; if necessary, special stains for bacteria and fungus may be done on the biopsy sample

    R/O SYRINGEAL GRANULOMA

Case Report #2

- Results
  - Histopathology:
    - Microscopic Description: biopsy specimen from the syrinx consists of an exudate of fibrin and degenerative inflammatory cells with fungal hyphae within the exudate; the fungi have narrow, septate branching hyphae

      Microscopic Findings: Fibrinous exudate, syrinx, mycotic

      Comment: The morphology of the fungal organisms in the exudate is consistent with *Aspergillus* sp.

  - Culture:
    - Aerobic & fungal cultures: no growth

Case Report #2

- 1-week post-operatively:
  - Doing well, vocalizing
  - Continue oral and nebulized medications
- 2-weeks post-operatively:
  - Continues to do well
  - Now screaming normally
  - Taper off oral meloxicam and enrofloxacin, nebulized medications
  - Continue oral itraconazole
- 4-weeks post-operatively:
  - Doing well
  - CBC, chemistry, serum protein EPH, *Aspergillus* antibody & galactomannan level all WNL
  - Continue oral itraconazole

Case Report #2

- 4-months post-operatively:
  - Doing well
  - Vocalizing normally
  - All medication stopped
- Results of CBC, chemistry, *Aspergillus* testing annually for 9 years have all remained WNL and bird is still symptom-free

Case Report #2

- This case demonstrates a common presentation of aspergillosis in a psittacine bird: tracheal granuloma
- All blood work was WNL despite extremely severe clinical signs
- For proper diagnosis and treatment of aspergillosis, need:
  - Rapid surgical intervention when birds present with life-threatening signs (i.e. upper airway obstruction) to get through acute phase of disease
  - Comprehensive diagnostic testing of surgical samples to ensure proper diagnosis
  - Long term treatment with antifungal drugs
  - Long term follow-up of cases to ensure bird doesn't relapse

Aspergillosis: Questions?