Miller, Michele A., et al. "Outbreak of Mycobacterium tuberculosis in a herd of captive Asian elephants (Elephas maximus): Antemortem diagnosis, treatment, and lessons learned." *Journal of Zoo and Wildlife Medicine* 49.3 (2018): 748-754.

Abstract: **Tuberculosis (TB) was diagnosed in four Asian elephants (*Elephas maximus*) in a zoo in the United States.** The first case was detected by **isolation of *Mycobacterium tuberculosis* during routine trunk wash (TW) culture** testing of a herd of eight elephants. Retrospective antibody analyses revealed **seroconversion 1 yr before** diagnosis. Serological testing of the whole elephant herd identified **two additional suspect bulls with detectable antibody, but which remained culture-negative** and had no clinical signs of disease. In the **following months, *M. tuberculosis*, identical to the isolate from the index case, was isolated from TW samples of these two** elephants. **A fourth elephant seroconverted nearly 4 yr after the first TB case was detected, and *M. tuberculosis* was isolated from a TW sample collected 1 mo later. All four infected elephants received anti-TB therapy. Two treated elephants were eventually euthanized for reasons unrelated to *M. tuberculosis* and found to be culture-negative on necropsy, although one of them had PCR-positive lung lesions. One infected animal had to be euthanized due to development of a drug-resistant strain of *M. tuberculosis***; this animal did not undergo postmortem examination due to risk of staff exposure. **The fourth animal is currently on treatment.** Serial serological and culture results of the other four herd mates have remained negative.

* Introduction:
  + Dx Mycobacterium tuberculosis in captive elephants often delayed due to infrequent collection of trunk wash samples, sporadic shedding, insufficient sensitivity of mycobacterial culture methods.
    - Serology useful for screening. Elephants with TB develop strong humoral responses.
    - Multiantigen print immunoassay (MAPIA) detected Ab in sera from positive elephants with majority having seroreactivity to antigens ESAT-6 and CFP10.
      * Same antigens included in the point-of-case dual path platform DPP test (DPPVetTB).
* Case series:
  + Infection confirmed in 4 captive Asian elephants in a zoo by positive TW culture.
  + All had antibody responses supportive of infection before detection of bacterial shedding. None had clinical signs.
  + Serologic test results can inform tx options and animal management strategies much earlier than diagnosis. Bacterial isolation required to confirm and for drug sensitivity.
  + Routine mycobacterial culture of annual triple TW samples performed historically.
    - **June 2013 positive bull elephant. 7 other elephants negative.**
    - DPP VetTB Ab test for that same elephant returned positive for antibodies as well as two other bulls.
    - **Retrospective testing of other elephants showed seroconversion of first elephant about 1 year before positive culture.**
      * Based on retrospective serology, suspected that two elephants in addition to the original positive were also infected.
    - **Increased TW sampling to monthly for the known positive and every 4 mos for the others.**
    - **Dec 2013 – Mucus sample in second elephant’s stall cultured positive. All three bulls persistent Ab reactivity on DPP and MAPIA.**
    - **March 2014 – positive TW sample from third elephant. This elephant seropositive in 2005.**
  + Treatments/outcomes:
    - First elephant – isoniazid, pyrazinamide, rifampin. Euthanized for progressive arthritis March 2015. One **caseous lesion in lungs consistent with infection. Culture negative but positive tissue PCR.**
    - Second elephant, third elephant – Same protocol.
      * Third elephant – Euthanized Dec 2015 for arthritis. **Cultured negative, retropharyngeal and axillary LN contained mineralized abscesses. Infection could not be confirmed with culture or PCR. Was acid-fast positive.**
      * Second elephant euthanized Feb 2017 – Drug resistant M tb.
        + Isoniazid, pyrazinamide, rifampin, levofloxacin attempted, poor patient compliance.
        + Subsequent **TW culture showed resistance.**
  + Fourth female cultured positive on TW in Feb 2017.
    - Retrospective – **Seroconversion 1 month prior to shedding.**
    - Tx isoniazid, pyrazinamide, ethambutol, rifampin.
  + **Elephants that are seropositive are strong suspects, prompt more close monitoring.**
    - **Elephants in any herd with confirmed infection should be monitored more frequently with TW cultures.**
    - **TW culture method is of poor sensitivity.**
    - **Tx may arrest shedding, but TW culture may not correlate with successful tx.**
    - **Serological assays were able to detect infection in these individuals relatively early despite the absence of active shedding and clinical dz, supports antemortem dx value.**
  + A human infection had casual contact with elephant 1 for about 1 hour, unlikely that there was direct human to animal transmission. Did not ID source of infection in this case series.
* **Takeaway: Serological screening, followed by an increased frequency of TW culture testing of antibody-positive individuals may facilitate earlier detection of M tb infected elephants, improving outcomes associated with treatment and minimizing risk to personnel and surrounding animals.**

Rivas, Anne E., et al. "Diagnosis and management of mycobacteriosis in a colony of little penguins (Eudyptula minor)." *Journal of Zoo and Wildlife Medicine* 50.2 (2019): 427-436.

Abstract: A group of zoo-housed little penguins (*Eudyptula minor*) was diagnosed with mycobacteriosis. **While undergoing multidetector computed tomography (MDCT) imaging for an unrelated research project, pulmonary lesions were detected in multiple individuals.** In general, birds appeared healthy and free of outward signs of disease. **After the loss of three individuals, polyclonal mycobacterial disease due to *Mycobacterium avium-intracellulare* complex was confirmed. Surviving birds were treated with rifampin (45 mg/kg), ethambutol (30 mg/kg), clarithromycin (10 mg/kg), and enrofloxacin (30 mg/kg) compounded into a single capsule administered once a day in food. After 3 mo of therapy, MDCT imaging documented a decrease in nodule size** and number in all remaining birds, with further improvement documented after 13 mo of treatment. MDCT imaging was invaluable for diagnosing disease, documenting disease progression over time, and assessing response to therapy. Early initiation of therapy before the development of outward signs of disease led to resolution of mycobacterial pulmonary lesions in multiple penguins. Mycobacterial disease in this group of little penguins, as well as previously published reports, suggests that the species is at increased risk for developing mycobacteriosis.

* Introduction:
  + Avian mycobacteriosis – Nontubercle or mixed-type lesions.
    - Aka Mycobacterium avium complex MAC or Mycobacterium avium-intracellulare complex MAIC.
      * Lipid-rich waxy cell wall.
    - Domestic fowl, sparrows, phesants, and partridges appear more at risk.
    - Anseriformes, Gruiformes, Galliformes in zoos.
    - Reports in Sphenisciformes – outbreak in little penguins at Henry Doorly Zoo.
* Case Series:
  + 15 male, 9 female little penguins, shipped from AUS to Bronx Zoo.
    - On arrival, quarantined 35 days.
    - Three fecals, viral testing for HPAI and Newcastle, standing rads.
  + Research project to assess normal respiratory system anatomy in little penguins with multidetector CT.
    - CBC, chem, EPH unremarkable.
    - Five penguins had nodular changes detected in the lungs, later confirmed as granulomas.
      * No clinical signs.
      * Repeated 6-8 mos after initial scans.
      * Progression in size and number of pulmonary lesions.
  + Mortalities.
    - 3yo male, acute dyspnea with audible tracheal wheezes.
      * Treated for suspected asperfillosis with oral terbinafine and itraconazole in addition to amphotericin B nebulization.
      * Dyspnea improved but did not fully resolve after air-sac cannula placement.
      * MDCT showed improvement in the pulmonary granulomas but luminal soft tissue densities resulting in tracheal occlusions present.
        + Died 85 days after initial presentation.
        + Necropsy – chronic granulomatous and geterophilic inflammation of air sacs, lungs, trachea, intralesional acid-fast bacteria, terminal bacterial sepsis likely cause of death.
    - 3yo male penguin
      * On first MDCT scan, large presumptive granuloma 75% left lung, not clinical.
      * Leukocytosis and monocytosis (46K and 11K).
      * Oral terbinafine and itraconazole started.
        + Granuloma continued to enlarge on subsequent imaging.
        + US guided FNA of granuloma showed positive acid-fast staining bacteria consistent with mycobacteriosis.
        + Treated with ethambutol, rifampin, clarithromycin, and amikacin, but bird was ultimately euthanized.
    - 3yo male penguin
      * Original MDCT showed a granuloma 10% left lung, later progressed to 75% left and 25% right lung.
      * Open-mouth breathing, euthanized.
  + Remaining birds
    - Gastric lavage (ventriculus), cloacal swabs, one case US guided FNA of lung granuloma before euthanasia, also sampled biofilms in the pool. All submitted for culture.
    - One case – gastric wash, cloacal swab, and lung aspirate all cultured M. intracelullare.
    - Other gastric washes and cloacal swabs cultured negative, including birds with nodules on MDCT scans.
      * Misc other Mycobacteria spp cultured from the pool sample, not considered pathogenic or significant.
    - Started on antimycobacterial cocktail – rifampin, ethambutol, clarithromycin, enrofloxacin.
    - Well tolerated.
    - Twice-weekly draining and scrubbing of the exhibit, with cleaning agents effective at disrupting mycobacterial cell walls implemented.
    - Bleach 100% effective at killing nontb myco at concentrations of 0.5% for 15 minutes. Once per week.
    - 4 months after initiating tx, repeated MDCT, all birds showed improvement. 13 mos later recheck, full resolution of pulmonary nodules in all but one affected bird. Last individual had size reduction but not complete resolution. Medical tx stopped in all birds except last one.
* Discussion:
  + Compounding meds into a single capsule administered once daily in food minimized cost and labor.
  + This antibiotic cocktail considered effective.
  + Environmental reservoirs usually required for collection outbreaks, bird-to-bird transmission has not been documented.
  + Inhalation of organisms also a possible route of infection.
  + Mycobacteria is hydrophobic and binds to air bubbles, common in areas with water aerosolization from waterfalls, fountains, and hose spraying. Also chronically wet, nutrient-rich soil-water interfaces.
  + Mycobacterial disease likely becomes active infection when immune system is suppressed. May be underreported when secondary ifnections i.e. aspergillosis are present.
  + MDCT imaging provided the most reliable means of dx for mycobacterial pulmonary lesions in little penguins in this report, as well as the best means to monitor response to therapy.
  + Culture remains gold standard despite low sensitivity and specificity.
* **Takeaway: Little penguins under human care are at risk for clinical mycobacteriosis, especially if stressed. Successful management depend son early diagnosis and tx. MDCT valuable for diagnosis and monitoring treatment.**

**Mycobacteriosis in captive psittacines: A brief review and case series in common companion species (Eclectus roratus, Amazona oratrix, and Pionites melanocephala)**

McRee, Anna Elizabeth, Higbie, Christine T., Nevarez, Javier G., Rademacher, Nathalie T., and Tully, Thomas N.

*Journal of Zoo and Wildlife Medicine* 2017;48(3): 851-858.

Abstract: In 2015, three psittacines were presented within 30 days, each with differing clinical signs and patient histories. A 13-yr-old male eclectus parrot (Eclectus roratus) was presented for weakness, depression, and acute anorexia. On presentation it was determined to have a heart murmur, severely elevated white blood cell count (93.9 103/ul) with a left shift (2.8 103/ul bands), and anemia (30%). Severe hepatomegaly was noted on radiographs, ultrasonography, and computed tomography. A cytological sample of the liver obtained through a fine needle aspirate revealed intracellular acid-fast bacilli identified as Mycobacterium avium. A 20-yr-old female double yellow-headed Amazon parrot (Amazona oratrix) was presented for a 1-mo history of lethargy and weight loss despite a good appetite. The parrot’s total white blood cell count was 16.8 103/ul and the PCV was 35%. Following its death, a necropsy revealed a generalized granulomatous condition that involved the small intestines, lungs, liver, spleen, and medullary cavities of the long bones, with intracellular acid-fast bacilli identified as Mycobacterium genavense. The third case, an 18-mo-old female black-headed caique (Pionites melanocephala), was presented with a 1-day history of lethargy and depression. On presentation, the caique had a heart murmur, distended coelom, palpable thickening of the coelomic organs, and increased lung sounds. Following the caique’s death, a complete necropsy revealed mycobacteriosis of the liver, spleen, small intestines, pericardial fat, and bone marrow. The infection was identified as Mycobacterium genavense. The importance of advances in Mycobacterium spp. identification, continued presence of this organism in captive avian populations, difficulty in obtaining a definitive antemortem diagnosis, and conflicting recommendations regarding treatment are thought-provoking areas of focus in this case series.

**Background**

* Most common psittacine species affected by mycobacteriosis: grey-cheeked parakeets, Amazon parrots, budgerigars, Pionus parrots
* Incidence in zoos 14-27% (all birds), 0.5-14% (psittacines)
* *M. genavense* more frequent than *M avium* subsp. *avium* in psittacines with advances in molecular differentiation
  + *Mycobacteruim avium* 4 subspp: *avium, hominissuis, paratuberculosis, silvaticum*
  + *M. genavense* also diagnosed in humans, cats with FIV, dogs, ferrets, dwarf rabbit, Mayotte Maki lemur, grizzled giant squirrel
  + Human to bird transmission with *M. tuberculosis* but not with *M. genavense*
* Fecal-oral transmission, shed in feces, persist in env for years, inhalation less common
  + Colonizes liver, small intestine, hematogenous spread
  + Diffuse enlargement of organs from macrophage accumulation, visible granulomas not always encountered
  + Down-regulates macrophages by preventing lysosome-phagosome fusion
  + Pathogenicity is specifically related to unique cell wall
* Usually individuals, population outbreaks often associated with immunosuppressive factors
* Classic disease has nonspecific signs and diagnostic results
  + Chronic wasting disease, polyphagia, weight loss, unkempt feathers, poor response to conventional antimicrobial therapy
    - Occasionally acute death with no clinical signs
    - Three phases: latency, lesion development, cachexia
  + May have marked leukocytosis with heterophilia, monocytosis, nonregenerative anemia
  + Imaging: enlarged cardiohepatic silhouette, thickened intestinal loops, increased opacity of endosteal bone in long bones
  + Acid-fast bacilli in fecal has ddx of nonpathogenic saprophytes
* Diagnosis confirmed by culture, isolation, genetic identification
  + Gold standard: biopsy of affected tissue for culture and nested PCR
  + Differentiated by sequencing unique 16S rRNA gene
  + Culture is slow growing: 1-6 mo for *M. avium*, *M. genavense* doesn’t grow on conventional mycobacterial media
  + Concentration > 10 viable organisms/ml required for positive culture
  + Intradermal tuberculin test has poor correlation with disease in psittacines
* Treatment options: isoniazid, rifampin, rifabutin, ethambutol, clofazimine, ciprofloxacin, enrofloxacin, streptomycin, amikacin
  + *M. genavense* successfully treated with clarithromycin in humans
  + Combination currently recommended due to rapid development of resistance to single drug protocols
  + Therapy at least 12-18mo: only killed during replication (every 16-20hr), persists in caseous lesions and macrophages for months
  + Hydrophobic cell wall restricts use of hydrophilic antibiotics (aminoglycosides, fluoroquinolones, macrolides)

**Key points**

* 3 cases presented to LSU within 30 days of varying species (*Eclectus roratus*, *Amazona oratrix*, *Pionites melanocephala*), age (18 mo to 20 y), sex, clinical duration (1d to 1 mo), bloodwork (normal to marked leukocytosis of 93k), clinical condition (normal to emaciated) and signs, and primary organ/system affected (liver, small intestine, bone).
* All three cases had mild anemia and cardiac changes (arrhythmia or murmur) and gene sequenced *M. genavense* (eclectus had mixed *M. genavense* and *M. avium*)
* All humanely euthanized due to zoonotic risk and poor prognosis
* Eclectus diagnosed antemortem on liver FNA – cytology, culture, PCR

**Conclusions**

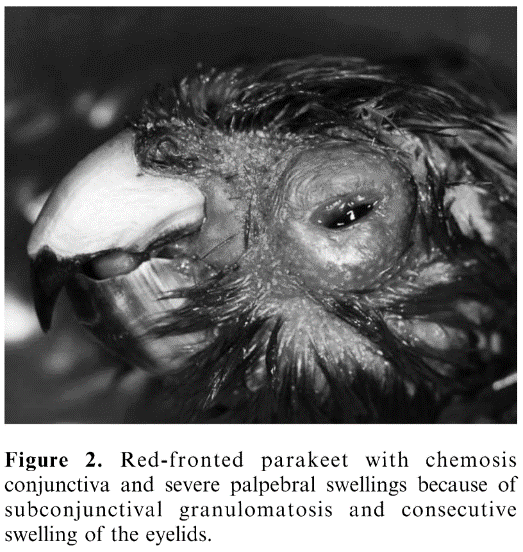
* Avian mycobacteriosis is a relevant disease that should be on the differential list for any insidious, nonspecific disease process
* Advances in spp. identification by gene sequencing shows increased prevalence of *M. genavense* in psittacines
* Continued effort needed in confirming an antemortem diagnosis and treatment options

**Retrospective evaluation of clinical signs and gross pathologic findings in birds infected with Mycobacterium genavense**

Anna Schmitz, Monika Rinder, Susanne Thiel Andrea Peschel, Kristina Moser, Sven Reese, and Rüdiger Korbel

*J Avian Med Surg.* 2018;32(3):194-204.

Abstract: *Mycobacterium genavense* is regarded as the primary cause of mycobacteriosis in passerine and psittacine birds kept in captivity. *Mycobacterium genavense* is a potential zoonotic pathogen; therefore, early antemortem detection in birds is needed. In humans, infections with *M genavense* are found predominantly in immunocompromised people. To investigate clinical signs and pathologic lesions and to determine the prevalence of coinfections in birds infected with *M genavense*, **we reviewed records of 83 birds in which DNA from *M genavense* had been detected via real-time polymerase chain reaction (Germany 2011-2016)**. To evaluate clinical signs in birds presented as patients, results of standardized examinations of 60 birds and radiographic results from 37 birds were investigated. Necropsy results of 82 of the 83 birds were evaluated, including results of additional parasitologic, bacteriologic, and virologic examinations. Birds included in the study comprised 15 species in the orders Passeriformes, Psittaciformes, Coliiformes, Columbiformes, Coraciiformes, and Ciconiiformes. A wide range of clinical manifestations were documented, including neurologic disorders, ocular manifestations, and gastrointestinal signs. Of the 60 birds examined clinically, 15% showed no clinical signs. Coinfections with a wide range of pathogens were detected in 52% (43 of 83) of the tested birds. Coinfections included *Macrorhabdus ornithogaster*, circovirus, polyomavirus, avian bornavirus, adenovirus, *Mycobacterium avium* ssp. *avium/silvaticum, Mycoplasma* species*, Salmonella* species*, Escherichia coli, Aspergillus* species, and various parasites*.* The high number of coinfections may reflect an impaired immune status in the birds examined. These results also suggest a broad host range for *M genavense*, and the existence of various clinical signs that may be strongly associated with coinfections with other pathogens.

****

**Background**

* *Mycobacterium genavense* difficult and slow to grow, zoonotic to immunosuppressed humans
* Clinical signs in birds: sudden death, nonspecific: weight loss, diarrhea, neurologic signs, alterations of eyelids, respiratory disorders
* Detected in feces of clinically healthy parrots
* PM nonspecific: hepatomegaly, lack of macroscopic foci may be characteristic in pet birds, +/- granulomas
* Sensitivity of PCR in past study: 105 bacteria per g feces, able to differentiate *M genavense* from other *M avium* subspp.

**Key Points**

* *M genavense* detected in 26 species in orders Passeriformes, Psittaciformes, Coliiformes, Columbiformes, Ciconiiformes
* Prevailing species: budgies, zebra finches, Eurasian goldfinches, vitelline masked weavers, red-fronted parakeets, canaries (\*sampling bias)
* 15% of infections had no clinical signs
* Passerines had better ‘general’ condition than Psittacines (no difference in BCS)
* Most common clinical signs: feather disorders, dyspnea, ocular disease, neuro (\*likely confounded by comorbidities)
* Rads most common: hepatomegaly, lungs and air sac shadowing, dilated intestines with contrast
* Path: 51% no significant gross findings, 21% white firm nodules
* 52% had coinfections, 2 fruit doves had coinfection with *M avium* subsp *avium*

**Conclusions**

* First report of *M genavense* in coliiform and ciconiiform birds – broad host range
* Passerines had better general condition than psittacines, Psittacines had more frequent hepatomegaly and granulomas
* Body condition, clinical signs, and necropsy findings can vary widely
* High rate of coinfection in this study pop may suggest immunosuppression was a factor

**MYCOBACTERIUM BOVIS IN FREE-RANGING LIONS (*PANTHERA LEO*) - EVALUATION OF SEROLOGICAL AND TUBERCULIN SKIN TESTS FOR DETECTION OF INFECTION AND DISEASE**

Michele A. Miller, Peter Buss, Tashnica Taime Sylvester, Konstantin P. Lyashchenko, Lin-Mari deKlerk-Lorist, Roy Bengis, Markus Hofmeyr, Jennifer Hofmeyr, Nomkhosi Mathebula, Guy Hausler, Paul van Helden, Eliza Stout, Sven D.C. Parsons, Francisco Olea-Popelka

J. of Zoo and Wildlife Medicine, 50(1):7-15 (2019)

**Taxonomy**: Mammalia → Carnivora → Felidae

**Abstract:** Bovine tuberculosis (bTB), caused by *Mycobacterium bovis* infection, causes morbidity and mortality in free-ranging lions in bTB-endemic areas of South Africa. However, the only currently used diagnostic test is the tuberculin skin test (TST). This test is logistically challenging to perform because it requires immobilization of lions twice in a 72-hr period. Blood-based diagnostic tests, such as serological assays, have been previously reported for *M. bovis* detection in lion populations, and have the advantage of only requiring a single immobilization. In addition, serological assays can be used for retrospective testing. Therefore, the aim of this study was to test free-ranging lions with the STAT-PAKt (Chembio Diagnostics Systems, Medford, NY 11763, USA) and DPPt VetTB (Chembio Diagnostics Systems) serological assays and compare those results with the tuberculin skin test. The serological assays were also used to determine prevalence in bTB-endemic and uninfected lion populations. The results showed that the serological assays could distinguish between M. bovis culture-positive and -negative lions. In addition, antigen-specific humoral responses were present in lions that had clinical signs of bTB disease or were shedding M. bovis antemortem. Although the seroprevalence of M. bovis infection in Kruger National Park lions was similar to that obtained from antemortem mycobacterial culture (4.8 and 3.3%, respectively), it was less than that estimated by the TST (72%). These findings support the hypothesis that assays based on cell-mediated immune responses are more sensitive than serology is in detecting M. bovis infection in lions. However, serological assays can have a role in bTB disease detection in lions and are especially useful for retrospective studies.

**Background:**

* *Mycobacterium bovis* is associated with mortality in lions in Kruger National Park
* Methods of transmission in lions:
  + Ingestion of infected prey
  + Aerosol
  + Contact
* Clinical signs: pulmonary and bone lesions, emaciation, death

**Discussion / Key Points:**

* 31 Kruger lions underwent nx - 52% confirmed M bovis on culture. If tracheobronchial lavage performed prior to culling then 100% agreement for TBL and culture post mortem
* Most culture positive lions were seropositive on STATpak and DPP vetTB and TST reactive
* Higher percentage of positives on STAT-PAK and TST in the culture positive group than the culture negative group.
* No culture negative Kruger lions were seropositive but some were TST reactive
* Most culture positive lions had chronic disease consistent with bTB
* Unexposed population was negative for culture, serology and TST; no disease for bTB on nx
* Nontuberulosis mycobacteria may cause false positive - small percentage of TBLs with NTMs (culture) identified were positive on STAT-PAK but not on DPP. Some NTM culture negative still had positive STAT-PAK
* STAT PAK sensitivity - 62.5% similar to other species

**Take Home Message:** STAT-PAK and DPP have a very high specificity (100% in this limited study). TST (cell mediated response) more sensitive than serology

**Related Articles:**

Sylvester TT, Martin LER, Buss P, Loxton AG, Hausler GA, Rossouw L, van Helden P, Parsons SDC, Olea-Popelka F, Miller MA. Prevalence and risk factors for Mycobacterium bovis infection in African lions (Panthera leo) in the Kruger National Park. J Wildl Dis. 2017;53(2):372–376.

Viljoen IM, Sylvester TT, Parsons SD, Millar RP, Helden PD, Miller MA. Performance of the tuberculin skin test in mycobacterium bovis–exposed and–unexposed african lions (panthera leo). Journal of wildlife diseases. 2019 Jul;55(3):537-43.

**Question:**

Which of the following is true regarding Mycobacterium bovis testing in free-raging lions in South Africa?  
**A) Post-mortem culture positive animals had 100% agreement for positive tracheobronchial lavage pre-mortem**

B) Culture negative lions were not tuberculin skin test positive

C) Nontuberculin mycobacteria caused positive results on DPP VetTB

D) Approximately one-fourth of the post-mortem tested population were culture positive for bTB

E) Culture positive animals do not typically show signs of disease on nx

**PERFORMANCE OF THE TUBERCULIN SKIN TEST IN *MYCOBACTERIUM BOVIS*–EXPOSED AND –UNEXPOSED AFRICAN LIONS (*PANTHERA LEO*)**

[Ignatius M. Viljoen](https://bioone-org.lp.hscl.ufl.edu/search?author=Ignatius_M._Viljoen), [Tashnica Taime Sylvester](https://bioone-org.lp.hscl.ufl.edu/search?author=Tashnica_Taime_Sylvester), [Sven D. C. Parsons](https://bioone-org.lp.hscl.ufl.edu/search?author=Sven_D._C._Parsons), [Robert P. Millar](https://bioone-org.lp.hscl.ufl.edu/search?author=Robert_P._Millar), P[aul D. van Helden](https://bioone-org.lp.hscl.ufl.edu/search?author=Paul_D._van_Helden), [Michele A. Miller](https://bioone-org.lp.hscl.ufl.edu/search?author=Michele_A._Miller)

JWD 55(3), 2019

**Taxonomy:** Mammalia → Carnivora → Felidae

**Abstract:** Lion (Panthera leo) populations, classified as vulnerable under the International Union for Conservation of Nature red list of threatened species, are facing a variety of threats, including tuberculosis (TB) caused by Mycobacterium bovis. The lack of knowledge on pathogenesis and diagnosis of TB, the prolonged course of the disease, the existence of subclinical infection, and nonspecific clinical signs hamper management of TB in both free-ranging and captive lion populations. Early and accurate antemortem diagnosis of M. bovis infections is important for disease management. In this study, we investigate the suitability of the single intradermal cervical test (SICT), developed with free-ranging Kruger National Park (KNP) lions exposed to M. bovis, for use in other lion populations. Using the recommended interpretation, the specificity of the SICT was low in disease-free captive lions, leading to false-positive diagnoses in 54% of individuals in the present study. Alternative interpretations of the tuberculin skin test are proposed that significantly reduce false-positive diagnosis in the sampled captive lions without significantly affecting diagnoses in the KNP lions; these changes may facilitate screening for M. bovis infection regardless of the exposure status of the lion population being investigated.

**Introduction**

* *Mycobacterium bovis* can cause significant mortality in lions - chronic, slowly progressive disease with non-specific clinical signs, hard to diagnose, hard to treat
* Single intradermal cervical test (SICT) developed using lions in Kruger National Park (KNP)
  + There is TONS of M. bovis there, all of these lions would have been exposed. Need to establish normal ranges of tests using unexposed animals.
* SICCT - single intradermal comparison cervical test - inject 2 sites, one with bovine purified tuberculin, one with avian, compare reactions - supposed to account for cross-reaction to non-tuberculous mycobacteria (NTM)

**Main points**

* Compared tuberculin skin test results from lions in KNP to lions not exposed to M. bovis (captive)
  + Results not specific enough for captive lions, false positives up to 54%
* SICCT reduced the number of false positives in captive lions
* Other tests for M. bovis:
  + Culture - relatively insensitive (cultured BAL samples)
  + Antigen-specific gene expression assay (GEA) - highly specific in lions, good comparison for tuberculin skin test results

**Conclusions:** Single intradermal cervical test is not specific in lions.

**Other literature:**

Mycobacterium Bovis In Free-ranging Lions (Panthera Leo) — Evaluation Of Serological And Tuberculin Skin Tests For Detection Of Infection And Disease. Journal of Zoo and Wildlife Medicine 50(1): 7–15, 2019

Sylvester TT, Martin LER, Buss P, Loxton AG, Hausler GA, Rossouw L, van Helden P, Parsons SDC, Olea-Popelka F, Miller MA. Prevalence and risk factors for Mycobacterium bovis infection in African lions (Panthera leo) in the Kruger National Park. J Wildl Dis. 2017;53(2):372–376.

Rosen, Laura E., et al. "Survey of antituberculosis drug administration and adverse effects in elephants in north America." *Journal of Zoo and Wildlife Medicine* 50.1 (2019): 23-32.

Abstract: Tuberculosis, caused by Mycobacterium tuberculosis, is a disease causing morbidity and mortality in captive elephants (Elephas maximus and Loxodonta africana) as well as free-ranging individuals. Elephants in North America diagnosed with tuberculosis are often treated with antituberculosis drugs, unlike livestock species, which has necessitated the development of treatment guidelines adapted from recommendations for humans. There are few published reports describing empirical treatment, which may be complicated by poor patient compliance, interruptions in drug administration, and adverse effects. A survey of elephants in North America was conducted to compile information on treatment protocols, including drugs, dosages, routes of administration, serum drug concentrations, and adverse effects of antituberculosis treatment. Responses were received regarding 182 elephants, 12 of which were treated prophylactically or therapeutically with antituberculosis drugs. Treatment protocols varied among elephants, and included various combinations of isoniazid, rifampin, pyrazinamide, ethambutol, enrofloxacin, levofloxacin, and ethionamide. Serum drug concentrations also varied considerably among and within individuals. Facility staff reported 5 elephants (out of 7 treated elephants with responses) that exhibited clinical signs that may have been associated with antituberculosis drugs or treatment procedures. Anorexia, decreased water intake, constipation, depression, ataxia, limb paresis, and tremors were among the signs observed. Most adverse effects were reported to be moderate or severe, resulting in interruption of the treatment. The results from this survey provide veterinarians and elephant managers with valuable historical data to make informed clinical management decisions regarding antituberculosis therapy in elephants.

Intro

* Infection with Mycobacterium tuberculosis (M. tb) is a growing disease concern for elephants
* Median prevalence 5.1% in Asian elephants in the US
* Diagnosis and treatment remain challenging
* Treatment of TB in elephants commonly uses combination therapy with the first-line drugs isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB)
* Determining doses is a challenge—PK studies have been done but no evaluation of efficacy
* Compliance and adverse effects pose additional challenges
* The objective of this study was to report the drugs, dosages, routes of administration, serum drug concentrations during therapy, and adverse effects from a larger pool of elephants under TB treatment to establish a baseline for the North American captive elephant population.

M&M

* A cross-sectional survey study was conducted to evaluate the diagnosis and treatment of TB in elephants in North America
* Two surveys were distributed: one on potential risk factors for TB, along with general information about history of TB and TB treatment, and one on TB diagnostic test results and treatment protocols.

Results

* 182 elephants, 162 of which had not been treated with anti-TB drugs
* Results based on 12 elephants from 5 facilities that reported treatment information, 8 of which also had demographic information available
  + All 8 were Asian elephants, 7 females, 1 male
* Of the 12 treated, 8 were prophylactically treated, 5 were treated therapeutically after a positive M tb culture, one treated both prophylactically and therapeutically
* The most frequently used drugs were INH (n = 12), PZA (n = 5), and enrofloxacin (n = 4)
* Large variation in doses and serum levels (when reported)
* No side effects were observed in 2 elephants. The other 5 of the 7 elephants with survey responses to this question experienced adverse effects. Four of these elephants experienced 2 to 5 adverse effects during treatment, with at least one adverse effect being reported by facility staff as moderate or severe (Table 4). For 4 of the 5 elephants, at least one adverse effect resulted in interruption of treatment.
* Most common adverse effect was anorexia (n=4), other common included decreased water intake (n=3) and depression-lethargy (n=3), which lasted 2 weeks or longer and often recurred. Ataxia reported in 2 individuals but did not recur

Discussion

* All Asian elephants
* Most treated prophylactically
* High variability in drug levels, though some of this may be due to artifact due to individual variation in metabolism (as demonstrated in humans with TB tx)
* Recommend monitoring serum concentrations of each individual throughout treatment
* Recommended concentrations based on efficacy from human literature
* Adverse effects are a concern for welfare and ability to maintain a treatment plan
* Treatment interruptions can create drug resistance and increase zoonotic risk

Brüns, Angela C., et al. "Diagnosis and implications of Mycobacterium bovis infection in banded mongooses (Mungos mungo) in the Kruger National Park, South Africa." *Journal of wildlife diseases* 53.1 (2017): 19-29.

ABSTRACT: Bovine tuberculosis (bTB) was first diagnosed in the Kruger National Park (KNP) in 1990. Research has since focused on the maintenance host, the African buffalo (Syncerus caffer) and clinically affected lion (Panthera leo). However, little is known about the role of small predators in tuberculosis epidemiology. During 2011–12, we screened banded mongooses (Mungos mungo) in the bTB highprevalence zone of the KNP for Mycobacterium tuberculosis complex members. Fecal swabs, tracheal swabs, and tracheal lavages of 76 banded mongooses caught in cage traps within a 2-km radius of Skukuza Rest Camp were submitted for Mycobacterium culture, isolation, and species identification. Lesions and lymph node samples collected from 12 animals at postmortem examination were submitted for culture and histopathology. In lung and lymph nodes of two banded mongooses, well demarcated, irregularly margined, gray-yellow nodules of up to 5 mm diameter were identified with either central necrosis or calcification, characterized on histopathology as caseating necrosis with epithelioid macrophages or necrogranuloma with calcified centre. No acid fast bacteria were identified with Ziehl– Neelsen stain. We isolated Mycobacterium bovis from lung, lymph node, and liver samples, as well as from tracheal lavages and tracheal swab from the same two banded mongooses. Blood samples were positive by ElephantTB STAT-PAKt Assay for 12 and Enferplexe TB Assay for five animals. Only the two banded mongooses positive on pathology and M. bovis culture were positive on both serologic assays. We provide evidence of bTB infection in banded mongooses in the KNP, demonstrate their ability to shed M. bovis, and propose a possible antemortem diagnostic algorithm. Our findings open the discussion around possible sources of infection and their significance at the human/wildlife interface in and around Skukuza.

Intro

* Bovine tuberculosis (bTB), caused by Mycobacterium bovis, has been one of the globally most important infectious diseases in cattle
* bTB was reported as early as the 1920s in South African wildlife
* African Buffalo as the maintenance host
* Lions most clinically affected
* small predators might contribute to the spread of bTB, similar to the European badger in the UK, but has not been investigated
* We evaluated whether the banded mongoose plays a role in bTB epidemiology in the Kruger National Park (KNP) and whether M. tuberculosis transmission at the small predator–human interface of rest camps occurs.

M&M

* 76 banded mongooses, within a 2-km radius of the restcamp in southwest KNP
* In 87 capture and recapture events, 76 individual banded mongooses were restrained in the cage traps and anesthetized
* Individuals were identified by ear notches and microchip transponders
* Selection criteria for euthanasia and subsequent postmortem examination were advanced age, a positive reaction on STAT-PAK, or presence of clinical signs typically associated with mycobacterial disease (n=12)
* Lymph node samples collected and submitted for histopath
* Laryngotracheal and fecal swab samples as well as tracheal washes (n = 73) were collected and submitted for mycobacterial culture and PCR
* Serology performed on live animals (n=74)

Results

* On postmortem examination, 1 of 12 animals had nine and one animal had one granulomatous lesion (lung and lymph nodes). Lesions were acid fast negative
* Culture - isolated M. bovis from 2 (3%) of 76 banded mongooses
* Various nontuberculous mycobacteria (NTMs) were isolated from 48 (63%) individuals, whereas no mycobacteria were recovered from 28 (37%) individuals
* The two M. bovis–infected banded mongooses were concurrently infected with members of five Mycobacterium groupings (M simiae, M avium, M szulgai, M parascrofulaceum and fortuitum)
* Serology - Twelve individuals (16%) were positive. only two individuals showed a very strong reaction
* The bTB status was evaluated statistically with the quantitative variables of age and weight and categorical variables location, signalment, health, and coinfection with NTMs.
* The only significant findings were that bTB-positive banded mongooses were older (P = 0.025) and weighed more (P = 0.008) than bTB-negative animals.
* In summary, two banded mongooses were antibody positive on all three serologic assays. These were the only two animals from which M. bovis was isolated from antemortem and postmortem samples and that had lesions of granulomatous character

Discussion

* Mycobacterial culture of ante- and postmortem samples confirmed that banded mongooses in the vicinity of the Skukuza Rest Camp in the southern KNP were infected with M. bovis.
* Mode of transmission likely aerosol due to lesions in lungs and head/neck LN’s, though oral can’t be ruled out as a second mode of transmission
* No percutaneous or nasal lesions seen
* Buffalo considered to be the most likely source of infection; mongoose scavenging may play a role in transmission
* Important implications for human/wildlife interface
* Serology - Only the two animals with macroscopic lesions yielded M. bovis on culture, both from lesions as well as antemortem sampled tracheal lavage and swab, suggesting that animals with negative antemortem Mycobacterium culture are false positive serologic test results.

We therefore concluded that only a combination of STAT-PAK or DPP with Enferplex interpreted in series seemed to identify M. bovis–infected animals correctly

**Mycobacteriosis in a Zoo Population of Chinese Gliding Frogs (Rhacophorus dennysi) Due to Mycobacterium marinum**

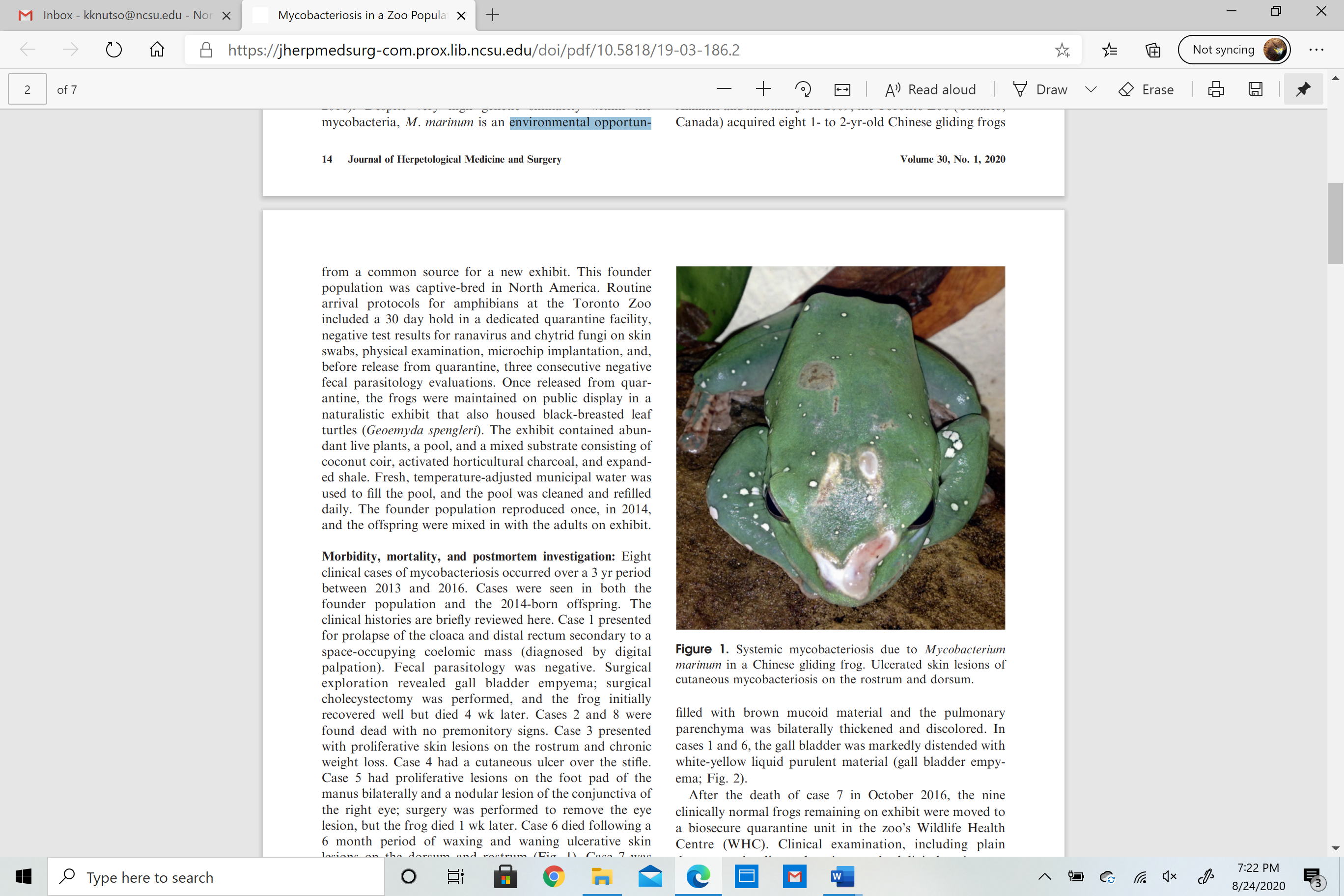
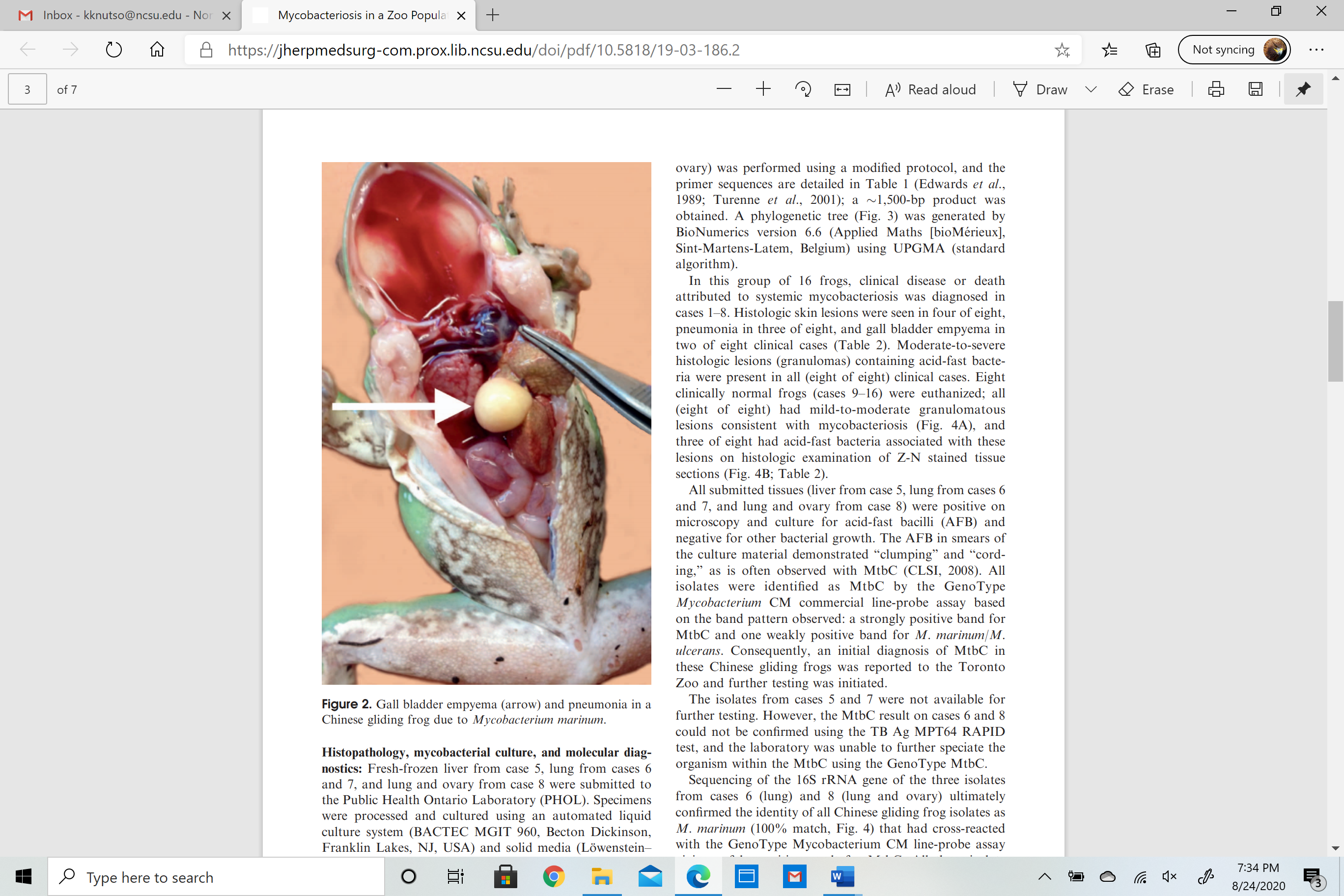
JHMS 2020 30(1) 14-20

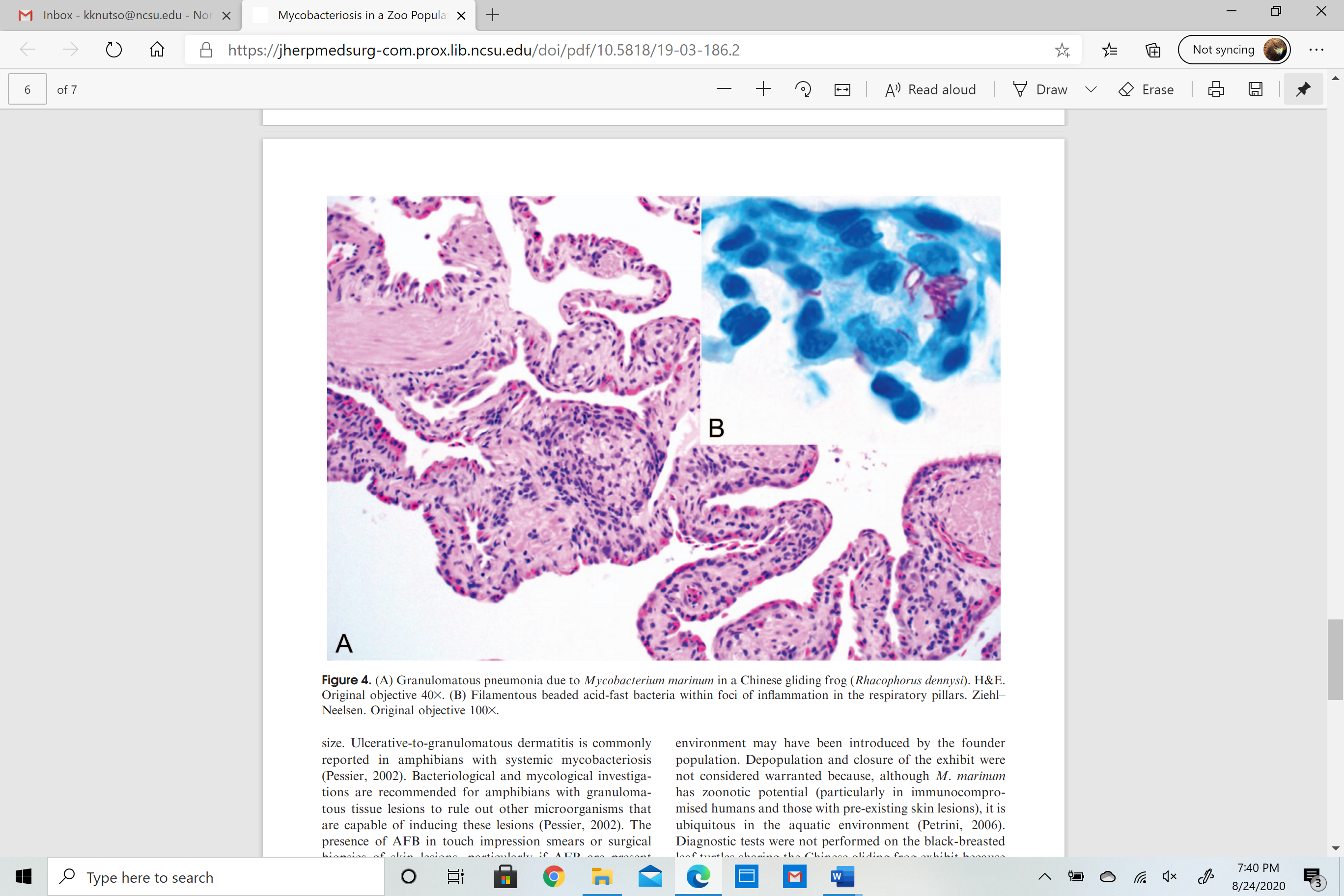
**Abstract:**

Mycobacteriosis was implicated in the deaths of eight Chinese gliding frogs (Rhacophorus dennysi) in a zoo population over a 3 yr period. Clinical signs included nonhealing skin lesions, cloacal prolapse, hind limb weakness, weight loss, and sudden death**. Abnormalities on postmortem were proliferative or ulcerative skin lesions in four of eight, pneumonia in three of eight, and gall bladder empyema in two of eight cases.** **All eight clinical cases had multisystemic granulomas containing acid-fast bacilli. Tissues most commonly affected were lung (seven of eight), liver (six of eight), kidney (six of eight), spleen (five of eight), and heart (five of eight).** The remaining eight clinically normal frogs in the population were euthanized: eight of eight had granulomatous lesions, with acid-fast bacilli in three of eight cases. A mycobacterial species was cultured from four of the clinical cases by the Public Health Ontario Laboratory and was initially misidentified as Mycobacterium tuberculosis complex by a commercial lineprobe assay (GenoType Mycobacterium CM, Hain Lifesciences, Nehren, Germany). Further diagnostic testing using 16S rRNA gene sequencing ultimately identified the mycobacterial species as Mycobacterium marinum. The correct identification of mycobacterial species is essential in epidemiological investigations at zoological facilities, and in assessing health risks to staff and to other animals in the zoo population.

**Summary:**

* Intro
  + Mycobacteriosis
    - important cause of morbidity and mortality in captive amphibians
    - most common isolates - Mycobacterium marinum, Mycobacterium fortuitum, Mycobacterium ulcerans, and Mycobacterium xenopi
    - Nontuberculous mycobacteria (NTM) common in aquatic environments
    - M. marinum
      * endemic in many zoo exhibits
      * environmental opportunist
      * zoonotic potential
* Case series
  + 8 cases of mycobacteriosis over 3 yr period
  + Case 1
    - presented for cloacal prolapse and distal rectum secondary to coelomic mass
    - surgical explore found gall bladder empyema 🡪 cholecystectomy performed
    - ceftaz post op
    - died 4 wk later
  + Case 2
    - Found dead
  + Case 3
    - Presented for proliferative skin lesions on rostrum and chronic weight loss
  + Case 4
    - cutaneous ulcer over stifle
    - Topical ofloxacin 1 wk – no improvement
    - died
  + Case 5
    - proliferative lesions on foot pad of manus bilaterally and nodular lesion of conjunctiva OD
    - surgical conjunctival lesion excision
    - ceftaz post op
    - died 1 week later
  + Case 6
    - 6 month waxing and waning ulcerative skin lesions on dorsum and rostrum
    - Topical ofloxacin 6 wk – no improvement
    - Acid fast organisms on skin impression smear
    - Died
  + Case 7
    - hind limb weakness a few days before found dead
  + Case 8
    - Found dead
* Discussion
  + GenoType Mycobacterium CM assay incorrectly assigned isolates of M. marinum to MtbC (false positive for M. tuberculosis complex)
    - ID ultimately determined by 16S rRNA gene sequencing
    - Use caution with this test
  + gold standard for diagnosis:
    - presence of granulomatous lesions and/or AFB on histopathology with ID of mycobacterial species by molecular techniques
  + ulcerative-to-granulomatous dermatitis - common in amphibians with systemic mycobacteriosis
  + antemortem test for small amphibians too small for imaging:
    - touch impression smears to look for AFB
    - surgical biopsies of skin lesions, particularly if AFB are present within the cytoplasm of phagocytic cells
  + culture or acid-fast stain from skin, feces, GIT of healthy amphibians may be incidental



**Mycobacterial disease and subsequent diagnostic investigations in a group of captive pinnipeds in New Zealand**

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**Abstract:**

This case series includes a single case of disseminated tuberculous disease due to Mycobacterium pinnipedii in a New Zealand fur seal (Arctocephalus forsteri), which was being cared for by a zoo in New Zealand. The remaining five pinnipeds in the colony underwent extensive mycobacterial disease surveillance over the following 4 yr, involving a total of 26 anesthetic procedures and numerous diagnostic tests that included comparative intradermal tuberculin skin tests, mycobacterial antibody serology, respiratory and gastric lavages, and computed tomography (CT) scans. An additional case of chronic sinusitis due to Mycobacterium marinum and Pseudomonas aeruginosa was identified in a California sea lion (Zalophus californianus). Results from CT and the respiratory lavages were the most helpful antemortem diagnostic tests for active mycobacterial disease in this case series. Of the remaining four animals, two were euthanatized and two remain alive, and none of them had evidence of active mycobacterial disease. Further mycobacterial disease surveillance in staff and animals was performed, and no other case was identified. There are no validated mycobacterial surveillance tests available for pinnipeds and so it remains unknown whether the two surviving pinnipeds are truly negative or whether they have latent mycobacterial infection that could develop into active mycobacterial disease in the future. For this reason, increased levels of biosecurity and quarantine remain permanently in place for the pinniped colony.

**Summary:**

* Intro
  + Mycobacterium pinnipedii
    - aerobic, nonmotile, slow-growing, acid-fast bacillus
    - belongs to Mycobacterium tuberculosis complex (M-TB)
    - closely related to Mycobacterium bovis
    - zoonotic
    - can be asymptomatic or only mild signs (g lethargy, weight loss, and anorexia)
  + Mycobacterium marinum
    - free-living, nontuberculous mycobacterium
    - found in freshwater and marine environments
    - common opportunistic pathogen of aquatic ectotherms
    - cutaneous granulomas in marine mammals and humans reported, occasional disseminated disease in marine mammals
    - self-limiting skin lesions in humans
* Case series
  + 6 pinnipeds – 3 New Zealand fur seals, 1 Subantarctic fur seal, 2 California sea lions
    - Case 1 – active Mycobacterium pinnipedii infection
      * ET tube flush positive for AFB on M-TB PCR and mycobacterial culture
      * BAL negative
    - Case 2 - Mycobacterium marinum with sinusitis, suspect secondary pathogen
      * Concurrent P. aeruginosa and P. mirabilis
      * BAL consistently negative M-TB PCR but positive for AFB and mycobacterial culture
    - Case 3-6 🡪 negative
* Discussion
  + Suspect case 1 had a latent infection, was originally wild
    - Highlights risk of accepting wild pinnipeds into permanent collection
  + serial lavage samples proved diagnostically useful in determining presence or absence of active mycobacterial infections
  + DPP Vet-TB
    - sensitive for detecting exposure to, or disease due to, M-TB bacteria
    - specificity extremely low
    - may help rule out active infection with M. pinnipedii or M. marinum
  + cITST
    - detects a cell-mediated immune response
    - low sensitivity and specificity for M-TB in validated species
    - cannot differentiate between latent and active infections
    - not validated in pinnipeds
    - false-negatives can occur with active disease
    - test of limited use during pinniped quarantine
  + **Mycobacterial culture** 
    - **gold standard test with 100% specificity**
  + M-TB PCR
    - similar specificity as culture and increased sensitivity
  + AFB stain
    - lower sensitivity and specificity than culture and PCR
  + Serology
    - confirms prior exposure
    - not ideal for diagnosing latent or active infection
  + **recommend performing tracheobronchial lavage and submitting sample to dedicated TB laboratory for AFB, M-TB PCR, and mycobacterial culture**
  + CT scans - useful in helping rule out active mycobacterial disease in cases 4–6
  + **Recommended combination of mycobacterial surveillance tests in pinnipeds:** 
    - **2+ tracheobronchial lavages (for AFB, M-TB PCR, and mycobacteria culture)**
    - **2+ DPP Vet-TB tests, taken at least 8 wk apart**
    - **1+ CT scan**
    - in the absence of positive tracheobronchial lavage culture, a positive DPP Vet-TB result or suspicious lesions on CT both have low specificity for active tuberculosis in this species