

Mycobacterium epizootic in a zoo population of Chinese gliding frogs (*Rhacophorus dennysi*)



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Chinese gliding frogs at the Toronto Zoo

- ▶ Foam-nesting tree frog native to China, Myanmar, Vietnam, and Laos
- ▶ Species of least conservation concern (IUCN 2004)
- ▶ Routine Toronto Zoo amphibian quarantine
 - ▶ 30 day hold in Wildlife Health Centre quarantine
 - ▶ Three parasite-negative fecal examinations
 - ▶ Physical examination
 - ▶ Microchip
 - ▶ Swab for *Batrachochytrium dendrobatidis* PCR
 - ▶ Ultrasound and radiography



Chinese gliding frogs at the Toronto Zoo

- ▶ Founder population acquired in 2009
 - ▶ Eight captive-bred frogs (one to two years old) from a US zoo
- ▶ Mixed-species naturalistic exhibit with black-breasted leaf turtles (*Geoemyda spengleri*)
- ▶ Sporadic deaths but no major health concerns
- ▶ Bred in 2014 → 22 offspring metamorphosed
 - ▶ 8 offspring moved to a US zoo in June 2014
 - ▶ 14 offspring remained at Toronto on exhibit with older cohort



2016 index case

- ▶ 2-year-old female
- ▶ Bred at Toronto Zoo (metamorphosed 2014)
- ▶ March 2016 ulcerative lesion on dorsum
 - ▶ Removed from exhibit for treatment
 - ▶ Impression smear: macrophages and toxic neutrophils, mixed bacteria
 - ▶ No bacteriology submitted
 - ▶ Healed over 4-6 weeks with topical treatment (hydrocolloid gel, ofloxacin) and systemic antibiotics (ceftazidime)
- ▶ May 2016 new lesions on rostrum and head, waxed and waned but never healed despite treatment
- ▶ Video camera set up in case of self-injury (glass collisions)
- ▶ Found dead 15 September 2016





July 2016

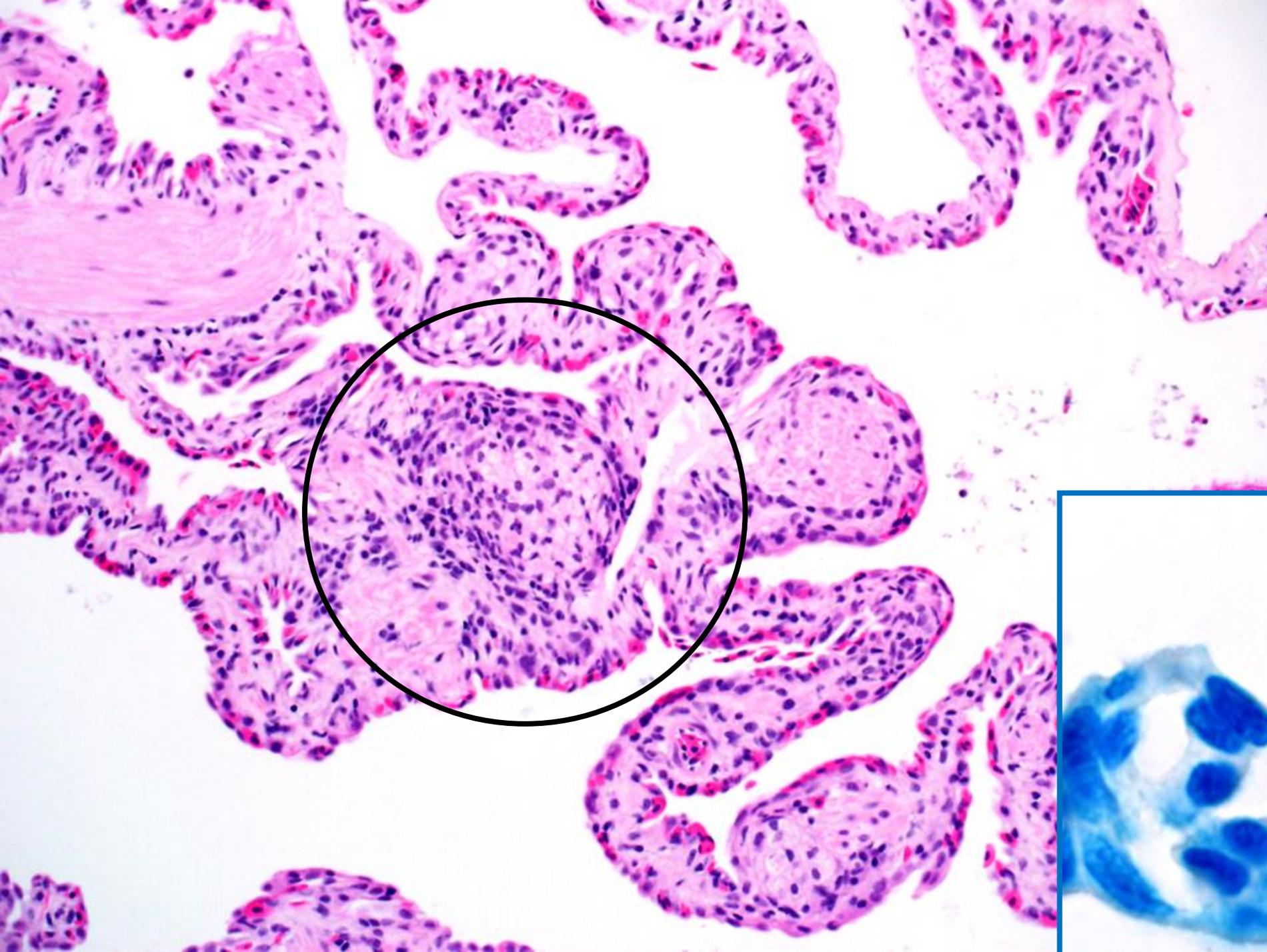


September 2016

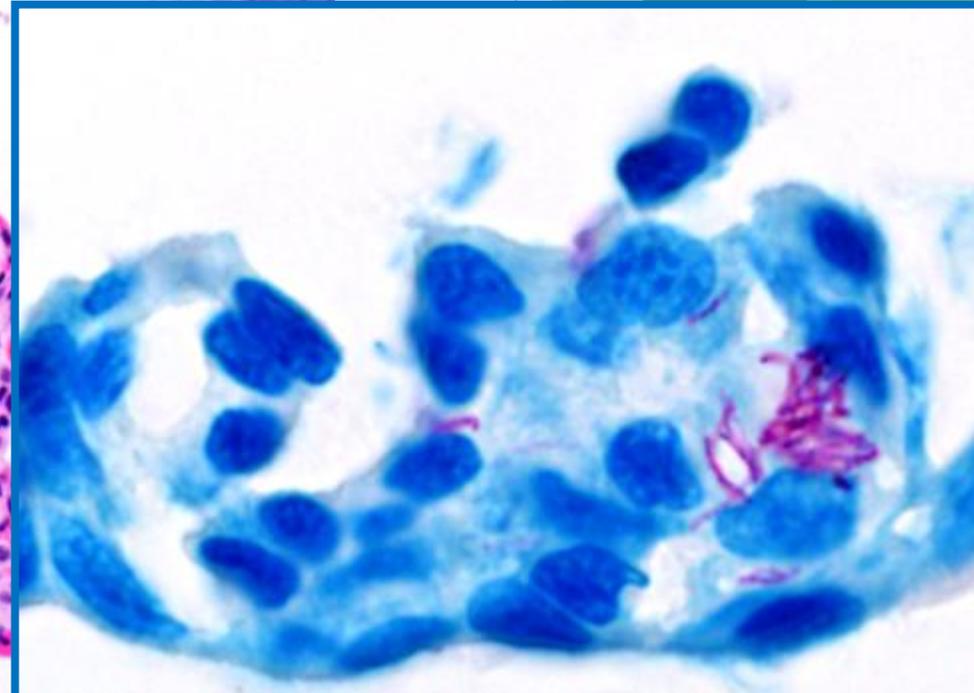
2016 index case gross necropsy

- ▶ Severe bilateral mucopurulent pneumonia
 - ▶ Lung parenchyma thickened and grey-brown (usually clear colourless membrane)
 - ▶ Airspace filled with brown mucus
- ▶ Immature ovarian follicles
- ▶ Hepatic lipidosis
- ▶ Atrophied fat bodies
- ▶ All tissues submitted for histopathology
- ▶ Lung sample frozen





Severe
granulomatous
pneumonia with
filamentous
beaded acid-
fast bacteria



Mycobacteriosis in amphibians

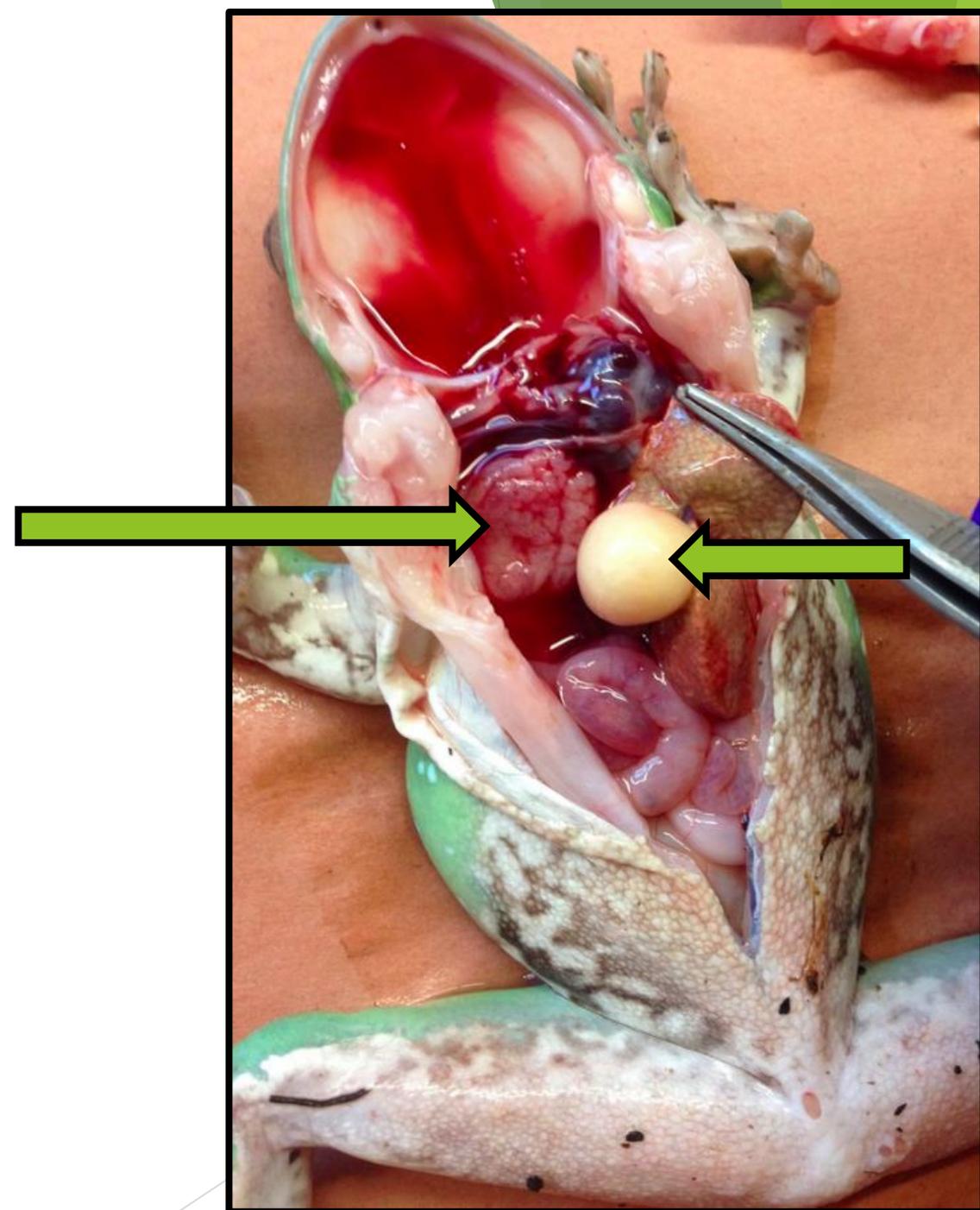
- ▶ Most zoos and aquaria occasionally experience amphibian losses due to non-tuberculous mycobacteria (NTM)
- ▶ Sporadic and epizootic morbidity and mortality in captive amphibians
 - ▶ Cutaneous ulceration, systemic granulomatous disease
 - ▶ Occasional NTM-related deaths at Toronto Zoo
 - ▶ Large die-offs in *Xenopus* laboratory colonies in Europe
- ▶ *M. marinum*, *M. fortuitum*, *M. xenopi*, *M. liflandii*
- ▶ No *M. tuberculosis* complex previously reported in any amphibian

Case 2: 4 October 2016

- ▶ 2-year-old unspecified sex found dead
- ▶ Discarded due to severe autolysis

Case 3: 28 October 2016

- ▶ 2-year-old male found dead
- ▶ Severe bilateral mucopurulent pneumonia
- ▶ Cholecystitis
- ▶ Atrophied fat bodies



Case 1: Animal Health Laboratory and Public Health Ontario results received 1 November 2016

Public health report, Mycobact Method ID:Public Health

Date Authorized: 2016-Nov-01 12:01

Sample ID	Client SampleID	Specimen type	Test	Result	Note
16-073628-0001	Z142-16	Lung	Result	3+ Acid fast bacilli seen on smear	
			Result 2	Mycobacterium tuberculosis complex	

Public health response at Toronto Zoo

- ▶ Public health response began immediately
- ▶ Staff nurse performed TB skin testing for all in-contact keepers and vets
 - ▶ No positive reactions, no illness
 - ▶ Several vet staff had childhood BCG vaccines so were not tested
- ▶ All remaining frogs and turtles in exhibit moved to biosecure quarantine in Wildlife Health Centre
- ▶ Access restricted to two trained keepers and vet staff wearing full PPE including N-95 masks

Further testing at Public Health Ontario

- ▶ 16S gene sequencing → novel strain of *Mycobacterium marinum* that had cross-reacted with the *Mycobacterium tuberculosis*-complex PCR.

Mycobacterium marinum

- ▶ Non-tuberculous mycobacterium (NTM)
- ▶ *M. marinum* sporadically reported in fish and amphibia in many zoos
- ▶ Natural transmission is poorly understood
- ▶ Experimental study in immunocompetent leopard frogs given a low dose of *M. marinum* developed chronic subclinical granulomatous disease → immunosuppressed with hydrocortisone → fulminant lethal mycobacteriosis (Ramakrishnan et al. 1997)

Zoonotic risk of *M. marinum* - November 2016 paper on pulmonary disease in an immunocompetent aquarist

Scott Med J. 2016 Nov;61(4):203-206. Epub 2016 Nov 21.

Pulmonary *Mycobacterium marinum* infection: 'fish tank granuloma' of the lung.

Velu PP¹, Fernandes SE², Laurenson IF³, Noble DD⁴.

⊕ Author information

Abstract

A 65-year-old man presented with a six-month history of lethargy, weight loss and dry cough. He had a background of mild chronic obstructive pulmonary disease. Chest radiograph showed new right upper lobe cavitary opacification. Sputum cultures were acid-fast bacilli smear positive and yielded *Mycobacterium marinum* - a non-tuberculous mycobacterium (NTM) often found in aquatic environments and rarely associated with respiratory disease. The suspected source was silent aspiration of contaminated water, likely due to his initiating the siphon of his fish-tank by mouth. He completed a one-year course of rifampicin, ethambutol and clarithromycin, with negative repeat sputum mycobacteria cultures and radiological improvement. This case report demonstrates a successful approach to investigation and further management of *Mycobacterium marinum* pulmonary disease - a rare condition, particularly in immunocompetent individuals, with limited treatment guidelines.

Database search: previously undetected epizootic in founder population

- ▶ Over 5,000 animals of 500 species at Toronto Zoo
 - ▶ Residents perform gross necropsy on all deaths
 - ▶ Histopathology screened with UoG pathologists
 - ▶ Pathology cases are prioritised by conservation concern
 - ▶ **Slow-burning epizootic in a low-conservation-concern species can be missed in a large collection**
- ▶ Internal database of zoo pathology cases (InMagic)

8 frogs arrived from USA



2009

2010

2011

2012

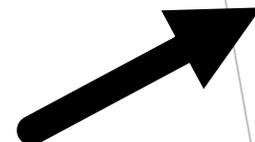
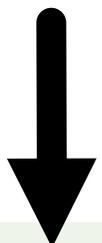
2013

2014

2015

2016

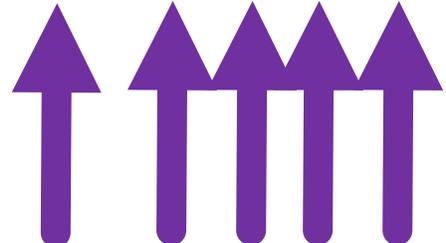
Bred, 22 frogs metamorphosed (2014 cohort)



8 frogs from 2014 cohort moved to zoo in USA, lost to follow-up

Founder population

8/8 died



= death not due to mycobacteriosis



= confirmed mycobacteriosis



= suspected mycobacteriosis

2014 cohort

8/22 transferred

5/22 died

9/22 in quarantine

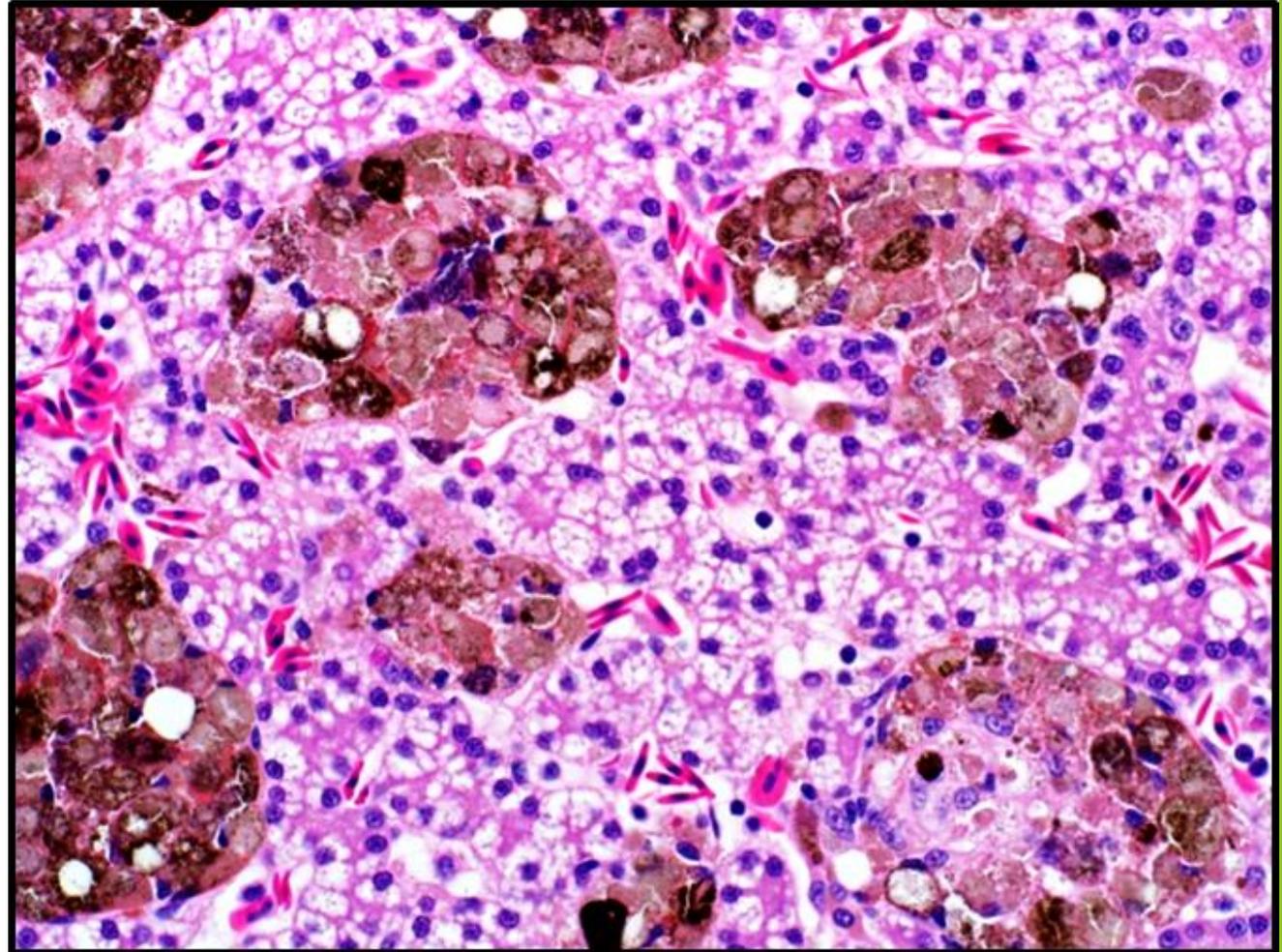


What to do with the frogs in quarantine?

- ▶ **Diagnosis of mycobacteriosis is difficult in living amphibians**
 - ▶ Requires histopathology + molecular species identification
- ▶ Clinical examination always difficult in frogs
- ▶ Radiography
- ▶ Endoscopic biopsy of coelomic organs
- ▶ Fourth frog found dead on 13 December 2016 → severe pneumonia and systemic mycobacteriosis despite appearing normal and under intense observation...
- ▶ Curator agreed to cull all remaining frogs

Necropsy findings for 9 euthanized frogs

- ▶ Systemic granulomatous disease of varying severity
- ▶ Granulomatous hepatitis (9/9), pneumonia (5/9), splenitis (4/9), pericarditis (3/9), nephritis (2/9), steatitis (1/9).
- ▶ Acid-fast bacteria (5/9)
- ▶ All appeared normal clinically and radiographically



Summary

- ▶ Initial identification of *M. tuberculosis* complex
 - ▶ Public health scare amongst keepers
- ▶ **New strain of *M. marinum* confirmed by 16S sequencing**
 - ▶ Highly pathogenic for Chinese gliding frogs
 - ▶ Black-breasted leaf turtles sharing the exhibit continue to thrive
 - ▶ Zoonotic concerns still present
- ▶ Unrecognized epizootic in founder population (2014-2014) identified with retrospective evaluation
- ▶ Radiology & physical examination - not useful for antemortem diagnosis

References and acknowledgements

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Ramakrishnan, L., Valdivia, R.H., McKerrow, J.H. and Falkow, S., 1997. *Mycobacterium marinum* causes both long-term subclinical infection and acute disease in the leopard frog (*Rana pipiens*). *Infection and immunity*, 65(2), pp.767-773.

Velu, P.P., Fernandes, S.E., Laurenson, I.F. and Noble, D.D., 2016. Pulmonary *Mycobacterium marinum* infection: 'fish tank granuloma' of the lung. *Scottish Medical Journal*, p.0036933016680161.

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