Slide 1

Slide 2


Kalumburu
Derby
Broome
Port Hedland
Roebourne
Exmouth
Australia
Western Australia
Carnarvon
Geraldton
Coolgardie
Perth
Eyre
Esperance
Albany
INDIAN OCEAN

Slide 3


Kanyana
• Respiratory syndrome
• Since the 1990s
• Admissions to Kanyana
• "Bobtail flu"
DONATE

Slide 4

'Bobtail Flu'

Clinical syndrome of:

- Sneezing
- Serous to mucopurulent nasal and ocular discharge
- Lethargy
- Inappetence
- Pallor of mucous membranes
- Depression
- Loss of body condition

- Syndrome termed Bobtail Upper Respiratory Tract Infection (URTI)

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Slide 5

C. Jackson c/o Kanyana

Slide 6

Slide 7

C. Jackson c/o Kanyana

Slide 8

C. Jackson c/o Kanyana

Slide 9

C. Jackson c/o Kanyana

Slide 10

‘Bobtail Flu’

- Anecdotal reports for 20 years
- Wildlife carers report increases over past 10 years (again this is anecdotal)
- 2015 – Kanyana Wildlife Rehabilitation Centre received approximately 200 bobtails, 40% of which were suffering from this disease
- Treatment consists of IM antibiotics, nebulisation, antiprotozoals and supportive care
- Approximately 85% of cases survive when admitted to care
- Unknown what happens to cases in the wild and released individuals

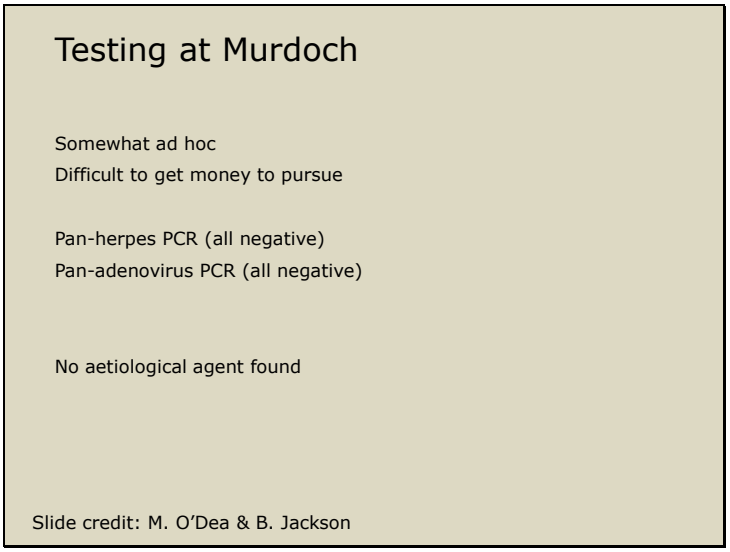
Slide credit: M. O’Dea & B. Jackson

Slide 11

Differential diagnoses

- Infectious
  - Viral*
  - Bacterial – typically secondary (*Mycoplasma agassizii*)
  - Fungal
  - Parasitic
- Non-infectious
  - Trauma
  - Neoplasia
  - Environmental pollutants

Slide 12



Bacterial culture? Post mortem examination?

Slide 13

2015 Study

- Cases entering Kanyana graded as acute, chronic or healthy
  - Acute – good body condition but serous/mucopurulent discharge
  - Chronic – poor body condition +/- discharge
  - Healthy – admitted for reasons other than URTI (ie animal attack)
- Single sample obtained from each case of an oral swab (FLOQswab™) placed into VTM and frozen
- 82 total cases in study
- 27 acute, 22 chronic, 33 healthy
- Aim: pan-pathogen screening/discovery using next generation sequencing

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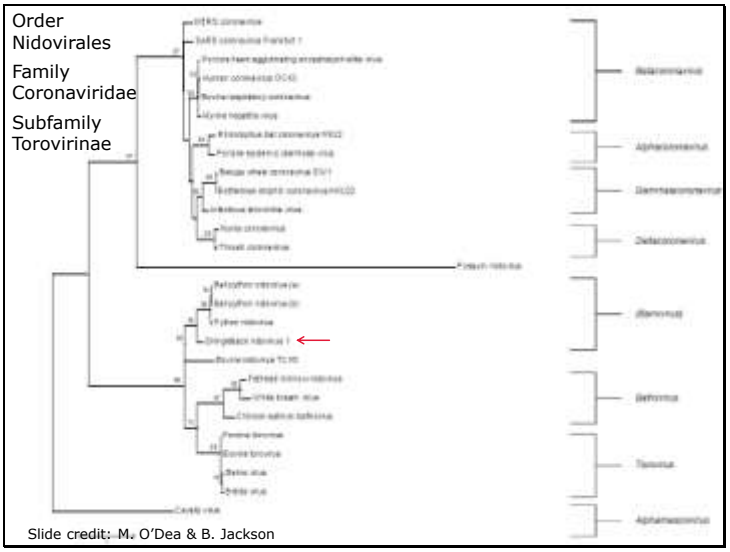
Slide 14



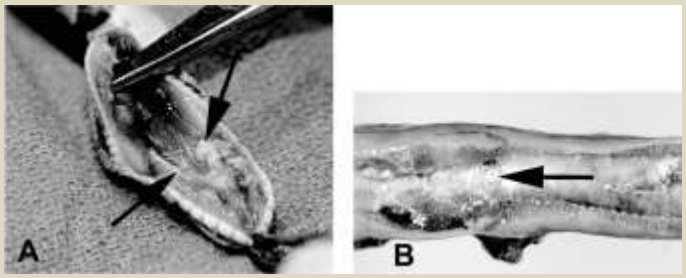
Refers to the order *Nidovirales*
Single-stranded positive sense RNA genomes
Viruses in the order *Nidovirales* have a number of classic genomic features including two large overlapping ORFs and a ribosomal frameshift site in the overlapping portion of the ORFs
Classified into the families *Arteriviridae*, *Coronaviridae*, *Mesoniviridae* and *Roniviridae*
Within the *Coronaviridae* are the coronaviruses and the toroviruses
The term nidovirus has been used to classify viruses which fall somewhere within the *Torovirinae* subfamily eg Possum nidovirus, Bovine nidovirus, Fathead minnow nidovirus

Slide 15

Python nidovirus

Ball python nidovirus – associated with severe respiratory disease in captive ball pythons (Stenglein et al 2014 mBio)

- Characterised by pharyngitis, stomatitis, sinusitis, tracheitis, proliferative interstitial pneumonia



(Stenglein et al 2014 mBio)

Slide credit: M. O'Dea & B. Jackson

Representative macroscopic lesions. (A) Common wild-type ball python (no. 1), *Python regius*. The palatine mucosa is both thickened and necrotic, and there is an accumulation of caseous material in the internal choanae. (B) Mojave variant ball python (no. 11), *Python regius*. The lung is thickened and edematous with abundant mucoid to caseous material (arrow) in air passageways. (Stenglein *et al.* 2014)

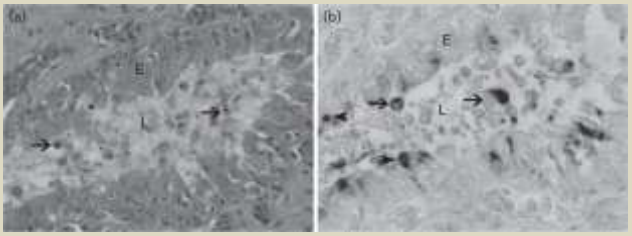
Slide 16

Python nidovirus

Indian python from a zoo with sudden death (Bodewes et al 2014 JGV)

Histology revealed severe, acute, necrotising pneumonia associated with mild inflammation and hypersecretion

Divergent nidovirus detected using 454 sequencing



H&E ISH

Slide credit: M. O'Dea & B. Jackson

Pathomorphological findings and *in situ* hybridization (ISH) in the lungs. (a) The pulmonary epithelium of the trabeculae (E) contains numerous necrotic cells and respiratory lumina (L) of the bronchi and faveolae are filled with necrotic cells (arrows) and cellular debris. Sections were stained with haematoxylin and eosin. Magnification ×400. (b) Detection of PNV RNA by means of ISH with evidence of positive viable cells (arrowheads) within the pulmonary epithelium (E) as well as of positive necrotic cells (arrows) within the lumen (L). Magnification ×400. (Bodewes *et al.* 2014)

Slide 17

Putting the puzzle together

We now have a potential viral candidate

It's closest match is to a virus causing respiratory disease in reptiles overseas (note however that these are significantly different)

It is within a viral family known for causing respiratory and gastrointestinal diseases

But is it the cause???

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Slide 18

Is it the cause?..results so far

Development of RT-PCR and testing of samples

Category	N	RT-PCR +ve	RT-PCR –ve	Prevalence (95% CI)	OR (95% CI)	p-value
Case	49	20	29	41% (27-56%)	5.0 (1.5-16.4)	0.006
Healthy	33	4	29	12% (3-28%)	NA	NA
Uncategorised	1	1	0	NA	NA	NA

- Quantitative RT-PCR – initial testing does not show correlation between cycle threshold (Ct) and case vs health (i.e. Positive "case" lizards do not carry more virus than positive "healthy" lizards)
- Pilot longitudinal study: virus can be detected in oral and eye swabs but not in cloacal swabs (i.e. doesn't appear to replicate in the gut)
- Lizards shed viral RNA for at least 4 weeks

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Slide 19

Initial histology

Only one sample so far submitted to Murdoch University Pathology as part of our work:

Trachea (A): One section of trachea is evaluated. The submucosa is multifocally infiltrated by rare lymphocytes, macrophages and heterophils.

Eyelids (B): Two sections of eyelids are evaluated. The conjunctival submucosa is expanded by low numbers of lymphocytes which exocytose through the overlying conjunctival mucosa. Adhered to the conjunctival mucosa is fibrillar eosinophilic material admixed with sloughed epithelial cells and cellular debris. The stratum corneum of the eyelids contains occasional colonies of 2-3um diameter cocci.

Not able to be ascribed to viral aetiology at this stage

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Slide 20

Plans

Discovery grant with Flinders and Curtin University

4 DVM students looking at the disease for their research projects

- Haematology/biochemistry
- Pathology
- Longitudinal shedding
- Diagnostic service

- Investigating the presence of this or related viruses in other reptile species

- Setting up a diagnostic service for this virus ($40/sample) - please contact Mark O'Dea or Bethany Jackson for further info

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Slide 21

Is this an emerging disease?

- Very hard to say
  - Anecdotal reports of increasing incidence
- Is there enough archived material (histology blocks) to look for historical presence?

Slide 22

References

- Bodewes *et al.* 2014. Novel divergent nidovirus in a python with pneumonia. JGV 95: 2480-2485.
- O'Dea *et al.* 2016. Discovery and partial genomic characterisation of a novel nidovirus associated with respiratory disease in wild shingleback lizards (*Tiliqua rugosa*). Plos One 11(11): e0165209
- Stenglein *et al.* 2014. Ball Python Nidovirus: a candidate etiologic agent for severe respiratory disease in *Python regius.* mBIO 5(5): e01484-14

Slide 23

Acknowledgements

New Tools For Old (And New) Diseases

A case study into 'bobtail flu'

Dr Mark O'Dea
Dr Bethany Jackson