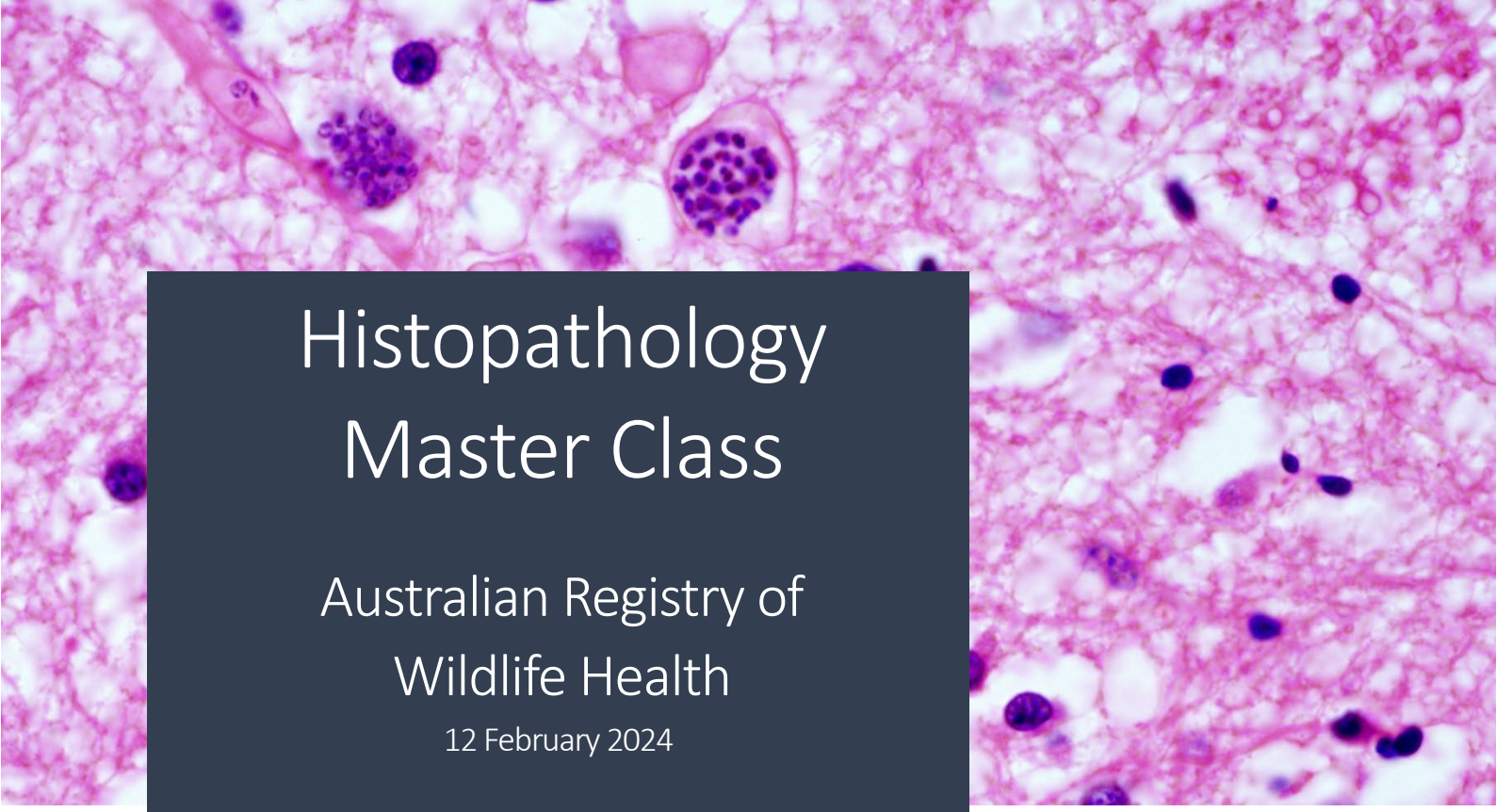

Histopathology Master Class

Australian Registry of Wildlife Health

12 February 2024

Presented by:
Dr Heather Fenton, Taronga Conservation Society
Dr María Forzán, Long Island University

8:30 – 9:00 am	Registration
	Seminar Room 1&2 Level 1 – Taronga Institute of Science and Learning
9:00 – 9:30 am	Welcome and Introduction
9:30 – 10:30 am	Group work on cases
10:30 – 11:00 am	Break
11:00 – 12:30 pm	Continued group work & individual work on cases
12:30 – 1:30 pm	Lunch
1:30 – 2:45 pm	Case presentations (Max 10 min each) Quokka: Nahiid Stephens Grey-headed flying-fox: Anthony Chamings Eastern grey kangaroo: Anne Jordan Tasmanian Devil: Graeme Knowles Ringtail Possum: Mark Hawes
2:45 – 3:00 pm	Break
3:00 – 4:00 pm	Remaining case presentations

Wildlife Health and Pathology Short Course 2024

Histopathology Masterclass

Case 1. Reptile A19-0133,3…… Contributor: Rob Ossiboff

Case 2. Reptile N19-524,14…… Contributor: Rob Ossiboff

Case 3. Northern Leopard Frog HE-X3743-04-2-1……. Contributor: María Forzán

Case 4. Northern Leopard Frog HE-X1810-09-3……. Contributor: María Forzán

Case 5. New Zealand Fur Seal TARZ3254.2……. Contributors: Jane Hall and Karrie Rose

Case 6. Tasmanian Devil TARZ 15459.1……. Contributor: Graeme Knowles

Case 7. Eastern blue tongue lizard TARZ 15297.1BB…… Contributor: Heather Fenton

Case 8. Green tree frog TARZ 14372.1…… Contributor: Heather Fenton

Case 9. Ringtail possum TARZ 15474.1…….. Contributor: Mark Hawes

Case 10. Red fox……. Contributor: Elizabeth Elsmo

Case 11. Red fox……. Contributor: Elizabeth Elsmo

Case 12. Flying Fox M18_1234_003 and M18_1234_004……. Contributor: Anthony Chamings

Case 13. Eastern Grey Kangaroo M22_14730,001,EW…….. Contributor: Anne Jordan

Case 14. Quokka AS-23-2377-2 and AS-23-2450{1..... Contributors: Rick Last and Nahiid Stephens

Case 15. Platypus ARWH 13294.1…….. Contributors: Jess Whinfield and Cheryl Sangster

Many thanks to all contributors for the excellent case material and to all participants for their interest in histopathology!

We also acknowledge the assistance of Mark Krockenberger and Elaine Chew from the University of Sydney for hosting the slides and assisting with making the slides available.

Dr. Peter Johnson and Jack Quinn were also instrumental in putting the workshop materials together.

CASE #1

Name: Robert Ossiboff, DVM, PhD, DACVP
Affiliation: Aquatic, Amphibian, and Reptile Pathology Program,
College of Veterinary Medicine, University of Florida
Gainesville, Florida, United States of America

Case Number: A19-0133N

History with Clinical Signs and Signalment: Juvenile (~8-month-old), captive bred, Brooks kingsnake (*Lampropelitis getula brooksi*) that is part of large collection of captive snakes where owners are noting high neonatal (<1 year of age) mortality in colubrid snakes. Affected snakes either continue to lose weight despite routine feeding, refuse food and lose weight, or regurgitate meals days after ingestion. Due to ongoing losses, animal euthanized for diagnostic investigation.

Gross Pathology: The snake was in thin body condition with diffuse atrophy (to loss) of coelomic fat bodies. The gastric rugae were extremely prominent (hyperplasia) and the stomach contained a moderate amount of mucus.

Microscopic Pathology: Both the gastric and duodenal mucosa are hyperplastic, inflamed, and studded with moderate to high numbers of apicomplexan protozoa (*Cryptosporidium* spp.). In the pyloric gastric mucosa, the lining mucosal epithelium is disorganized with nuclear piling, and is associated with low numbers of lymphocytes, plasma cells, and granulocytes; there are also low numbers of individual necrotic epithelial cells. Studding the apical surface of the gastric epithelium and extending down into gastric glands are moderate numbers of 3-6-micron diameter, basophilic parasites. Multifocal gastric glands are tortuous and increased numbers of granulocytes, lymphocytes, plasma cells, and fibroblasts are present within the lamina propria. In the duodenal mucosa, there is moderate to marked mucosal hyperplasia associated with moderate numbers of intraepithelial lymphocytes and fewer plasma cells and granulocytes. Very high numbers of apical brush border oriented cryptosporidial parasites, 2-7-micron in diameter, are present along the full length of the intestinal villi. Moderate numbers of lymphocytes, plasma cells, granulocytes, and fewer histiocytes are present in the intestinal lamina propria and are accompanied by moderate hyperplasia of associated lymphoid tissue aggregates. There is diffuse and marked pancreatic zymogen granule depletion and mild, multifocal atrophy of coelomic adipose aggregates.

Ancillary Tests: A sample of frozen gastric mucosa was positive for *Cryptosporidium serpentis* by qualitative PCR and subsequent Sanger sequencing. A sample of frozen intestine was PCR positive for mixed cryptosporidia (a dominant signal of *Cryptosporidium* sp. kn732 and an underlying signal of *C. serpentis* presumed to represent gastric pass through) as determined by Sanger sequencing.
Disease name: Cryptosporidiosis, concurrent gastric and intestinal

Discussion and Significance:

- Cryptosporidial infections are an important cause of morbidity and mortality in captive reptiles. While infections of free-ranging reptiles have been observed in North America, their clinical significance is unclear.
- While gastric cryptosporidiosis is most common in captive snakes, and captive enteric cryptosporidiosis is most common in lizards, both forms can be found in all captive squamates.
- Cryptosporidial species have specific tissue tropisms. In snakes and lizards, *C. serpentis* is the most common cause of gastric disease, while *C. varanii* is the most common cause of intestinal disease. However, other cryptosporidia are known to infect and cause disease in captive squamates.

- In individuals with both gastric and intestinal infection, both digestion and absorption of nutrients are impaired, and clinical disease can progress rapidly.
- In this case, *C. serpentis* was identified from the stomach, and the presumed agent in the intestine is a parasite species first described Japanese grass snakes (sp. kn732).[1]

References

[1]Kuroki T, et al. 2008. Occurrence of *Cryptosporidium* sp. in snakes in Japan. Parasitol Res.103(4):801-5.

CASE #2

Name: Robert Ossiboff, DVM, PhD, DACVP
Affiliation: Aquatic, Amphibian, and Reptile Pathology Program,
College of Veterinary Medicine, University of Florida
Gainesville, Florida, United States of America

Case Number: N19-524,14

History with Clinical Signs and Signalment: An adult, female, free-ranging gopher tortoise (*Gopherus polyphemus*) was submitted to the Zoo and Wildlife Medicine clinical service at the University of Florida lethargic and depressed, unable to open its eyes, and in thin body condition with bilateral nasal discharge. The tortoise was euthanized due to quality of life concerns.

Gross Pathology: The gopher tortoise was in extremely thin body condition with muscle and fat atrophy. Both eyes were sunken and there was concave deformation of the nares bilaterally with a small amount of mucoid discharge.

Microscopic Pathology: The nasal mucosa is diffusely inflamed and hyperplastic with areas of erosion. High numbers of granulocytes and lymphocytes are present within the hyperplastic nasal mucosa. The submucosa is expanded by clear space (oedema), and infiltrates of lymphocytes, plasma cells, granulocytes, and fewer histiocytes often surrounding submucosal vessels lined by reactive endothelial cells and nasal glands that are similarly inflamed as the nasal mucosa. Nodular accumulations of lymphocytes and plasma cells (mucosa-associated lymphoid tissue) are prominent in the submucosa. In the lumen of the nasal cavity, there are accumulations of granulocytes, cellular debris, and mucus admixed with myriad spiral shaped bacteria (*Helicobacter*-like organisms). Similar bacteria are adhered to the surface of the nasal mucosa and multifocally accumulating in the lumen of nasal glands and rarely extending into the glandular and surface mucosa.

Ancillary Tests: PCR and Sanger sequencing of the 16S rRNA gene on a sample of nasal discharge resulted in a 1391 base pair product of a Helicobacter species distinct from all available species with available sequence in GenBank. No *Mycoplasma* species were detected. Additional genes, DNA gyrase A subunit (*gyrA*) and chaperonin (*groEL*), were also amplified and sequenced, and results were consistent with a novel *Helicobacter* sp. of gopher tortoises.

Disease name: Nasal helicobacteriosis

Discussion and Significance: Bacteria of the genus *Helicobacter* are found in diverse host species, including reptiles, but the clinical implications of *Helicobacter* infections in tortoises are not well understood. This case is one of a group of cases in which a novel mortality-associated Helicobacter species was identified in free-ranging and rehabilitating gopher tortoises (*Gopherus polyphemus*) in Florida, United States.[1] Histologic changes associated with the gopher tortoise *Helicobacter* species

predominantly manifested in the nasal cavity. Spiral-shaped organisms were plentiful in this individual (as highlighted by Warthin-Starry staining). However, this tortoise did not receive antemortem antimicrobials. In tortoises with a history of antimicrobial therapy, while similar nasal inflammation was noted histologically on postmortem examination, spiral shaped bacteria were rare to absent. In tortoises with suspicious clinical signs for nasal helicobacteriosis and a history of antimicrobial therapy, PCR and/or cytologic evaluation of nasal discharge may be critical for a definitive diagnosis. While no *Mycoplasmopsis* (*Mycoplasma*) was detected in this individual, *Mycoplasmopsis* should always be considered as a differential in tortoises with upper respiratory tract disease. Evaluation of the nasal mucosa is an essential component of a tortoise necropsy, and samples should always be evaluated histologically. The author has observed *Helicobacter*-like organisms in other chelonians with ocular/nasal inflammation, and while characterization is ongoing, it may represent an important differential for chelonians with upper respiratory and ocular inflammation.

References

[1]Desiderio TM, Stacy NI, Ossiboff RJ, Iredale M, Archer LL, Alexander AB, Heard DJ, Crevasse SE, Craft WF, Fredholm DVE, Donnelly KA, Rosenberg JF, Childress AL, Russell K, Wellehan JFX Jr. Identification of a novel mortality-associated *Helicobacter* species in gopher tortoises (*Gopherus polyphemus*), qPCR test development and validation, and correlation with mortality in a wildlife rehabilitation population. Vet Microbiol. 2021 Aug;259:109136. doi: 10.1016/j.vetmic.2021.109136. Epub 2021 Jun 18. PMID: 34214906.

CASE #3

Name: Dr María Forzán
Affiliation: Long Island University College of Veterinary Medicine
720 Northern Boulevard
Brookville, NY 11548, USA

Case Number: X3743-04-2-1

History with Clinical Signs and Signalment: This wild-caught northern leopard frog (*Rana* [*Lithobates*] *pipiens*) from eastern Canada was one of three individuals euthanized at an ecotoxicology laboratory due to poor health. The frogs had been in the laboratory for less than 12 months, in the hope of producing offspring for pesticide impact studies.

Gross Pathology: This was a female leopard frog. Epidermal erosions and ulcerations approximately 5 mm in greatest diameter were present bilaterally on the palmar surface. The liver was diffusely dark green. The right lung was markedly expanded and filled with air until approximately 6-8 times the size of a deflated lung. Both lungs contained numerous small (3-4 mm-long) nematodes and several plump black and white non-segmented helminths (probably trematodes). The spleen was enlarged, approximately 2-3 times its normal size, with normal consistency.

Microscopic Pathology: In the air spaces of the lungs there are several cross-sections of metazoan parasites. The majority of the parasites have a bright eosinophilic cuticle around a pseudo coelomic cavity that contains vacuolated lateral cords, a reproductive and digestive tract, the latter with a tri-radiate oesophagus (adult nematodes, probably *Rhabdias* sp). A few small larvae are also present within the parenchyma. Fewer larger helminths are found in the air space of one of the lungs; these larger parasites have a solid (non-segmented) body covered with spiny cuticle, large uteri filled with brown thick-shelled eggs, large testes with spermatozoans at various stages of development, subcutical glandular tissue (interpreted as vitellaria glands) and symmetrical tubular organs (interpreted as paired ceca) (consistent with trematodes, possibly *Alaria* sp.).

In the ovary, a few histiocytic granulomas with a core of necrotic debris rich in melanin pigment are found (interpreted as regressing follicles). The mesovarium and adjacent mesentery also contain large areas of granulomatous and heterophilic inflammation.

The pericardium is diffusely thickened by an infiltrate of mononuclear inflammatory cells, mostly lymphocytes and macrophages, admixed with occasional heterophils. Numerous clusters of similar mononuclear inflammation, including cells containing a few melanin granules (melanocytes?), are found in the subepicardium and scattered throughout the myocardium. A severe multifocal to coalescing mononuclear infiltrate is present the wall of the aorta, often extending into the intima, media, and adventitial layers (transmural). The mesentery attached to the pancreas also contains lymphohistiocytic infiltrates. The inflammatory cells often extend into the adjacent interstitium, expanding the space between exocrine acini.

Additional findings in slides not shown: The serosa of the intestine is raised by the presence of numerous submesothelial foci of lymphohistiocytic inflammation, with lesser numbers of scattered heterophils. Numerous small aggregates of lymphocytes and macrophages, often along with melanocytes, are scattered throughout the hepatic parenchyma. Histiocytic granulomas are multifocally present in the kidney.

The palmar surface of one of the fore limbs has a large focal epidermal ulceration. The exposed dermis is markedly infiltrated and expanded by lymphocytes and macrophages along with occasional heterophils. The inflammation extends into the adjacent skeletal muscle, distending the perimysium between muscle bundles.

Ancillary Tests: Liver tissue was submitted to Prairie Diagnostic Services (Saskatoon, Canada) for Iridovirus PCR testing.

Disease name: Ranavirosis; pulmonary nematodiasis and trematodiasis (incidental)

Discussion and Significance (WOAH list, Zoonotic, notifiable): Ranavirosis is one of three amphibian diseases notifiable to the WOAH (formerly OIE).[4] It can cause severe disease in adults in captivity, and up to 100% mortality in tadpoles either captive or free-living.[1] Infection with helminth parasites, both *Rhabdias* and *Alaria* spp, is not uncommon in wild-caught individuals and likely an incidental finding unless re-infection in captivity results in high infection intensities.2,3

References

[1]Miller DL, Pessier AP, Hick P, Whittington RJ, Forzán MF. Pathology and Diagnostics, In: Ranaviruses, Lethal Pathogens of Ectothermic Vertebrates, 2024, in e-press (ranavirus.org)

[2]Kuzmin Y, Tkach VV, Snyder SD. The nematode genus *Rhabdias* (Nematoda: Rhabdiasidae) from amphibians and reptiles of the Nearctic. Comparative Parasitology. 2003 Jul;70(2):101-14.

[3]Lemke LB, Dronen N, Fox JG, Nambiar PR. Infestation of wild-caught American bullfrogs (*Rana catesbeiana*) by multiple species of metazoan parasites. Journal of the American Association for Laboratory Animal Science. 2008 May 8;47(3):42-6.

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CASE #4

Name: Dr María Forzán
Affiliation: Long Island University College of Veterinary Medicine
720 Northern Boulevard
Brookville, NY 11548, USA

Case Number: X1810-09-3

History with Clinical Signs and Signalment: This wild-caught northern leopard frog (*Rana* [*Lithobates*] *pipiens*) from eastern Canada was kept in an ecotoxicology laboratory in the hope of producing offspring for pesticide testing and had been used for breeding for two years. The frog had been kept in multiple tanks and had received previous injections. It was euthanised after appearing stressed and lethargic with irregular breathing. A swollen abdomen and possible sores on its digits were also noted.

Gross Pathology: The frog was emaciated, and multiple, small, dark foci were present within the spleen. There were no other significant findings.

Microscopic Pathology: The spleen contains fairly well-defined clusters of macrophages (granulomas). On the mesenteric surfaces of the viscera, including the intestine, liver, testes and heart, there are fairly large clusters of macrophages that rarely contain intracytoplasmic acid-fast bacteria. In the liver, the hepatic cords seem compressed by clusters of mononuclear cells, some with a band-shaped nucleus, occasional ones with eosinophilic cytoplasmic granules, but most organized in compact clusters and containing abundant black-green pigment that often obscures their nuclei (macrophages in pigment granulomas). In the atrial wall of the heart, at the junction with the ventricle, there is a focus of histiocytic inflammation similar to those in the mesentery. In the kidney, there are a few tubules whose lumina has been expanded and obliterated by many concentric layers of keratin (squamous metaplasia of the renal tubular epithelium). Several tubules have an attenuated epithelium and are filled with hypereosinophilic and glassy (protein-rich) fluid.

Ancillary Tests: Ziehl Neelsen (ZN) stain & PCR on formalin-fixed, paraffin-embedded (FFPE) tissue
A Ziehl Neelsen stain highlights the presence of a few rod-shaped acid-fast organisms up to 5 microns in length and sometimes with a beaded appearance within the cytoplasm of macrophages in multiple tissues.

Disease name: Mycobacteriosis

Discussion and Significance: This bacterium is potentially zoonotic.[4] *Mycobacterium marinum* is the most common species associated with infections in amphibians although other species have been reported.[3,4] This bacterium almost exclusively is a disease of captive amphibians with severe infections and outbreaks associated with husbandry and environmental contamination.[1,3,4,5] Historically, infections in captive *Xenopus laevis* were thought to be a 'transmissible lymphoma'.[5] Confirmation is often with culture, molecular methods and ZN staining.

References

[1]Asfari M. (1988). Mycobacterium-induced infectious granuloma in *Xenopus*: histopathology and transmissibility. Cancer research. 48:958-63.
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[5]Ramakrishnan L, Valdivia RH, McKerrow JH, Falkow S. (1997) *Mycobacterium marinum* causes both long-term subclinical infection and acute disease in the leopard frog (*Rana pipiens*). Infection and immunity. 65:767-73.

CASE #5

Name: Jane Hall / Dr Karrie Rose
Affiliation: The Australian Registry of Wildlife Health
Taronga Zoo, Bradleys Head Road
Mosman NSW 2088

Case Number: TARZ-3254_1

History with Clinical Signs and Signalment: This subadult, male, free-ranging New Zealand fur seal (*Arctocephalus forsteri*) was brought into rehabilitation as an emaciated and injured animal with suspected shark predation. During repeated examinations during its time in rehabilitation, several small, raised, and coalesced plaques were present on the roof of the mouth. Smaller raised plaques were present under the right side of the tongue and near the base of the teeth. A few lesions were present under the left side of the tongue. A biopsy of the oral mucosa was submitted.

Microscopic Pathology: The central section of the epithelium was markedly thickened and formed an array of papillary folds. This epithelium was covered by a thick layer of loose keratin. The epithelium appears to be primarily thickened due to an increased number of spinous epithelial cells with abundant basophilic cytoplasm. Moderate numbers of cells with pyknotic or karyorrhectic nuclei were scattered throughout this region of the epithelium. Moderate numbers of cells near the stratum basale were binucleate. Multiple foci of intracellular oedema and vacuolation were present occasionally with pyknotic nuclei (koilocytes). The spinous processes between epithelial cells were most prominent throughout the thickened layer of the epithelium. There was a discrete demarcation between the normal and abnormal epithelium. Within the proliferative section of epithelium these cells are markedly basophilic and large compared with the more normal region of epithelium.

Ancillary Tests: *Brucella* sp. serology was negative. Metatranscriptomic analysis and subsequent rt-PCR testing identified a novel papillomavirus from oral tissue that had been frozen at -80^{o}C for 20 years.

Disease name
Oral Papilloma Virus (provisionally termed *Arctocephalus forsteri* papillomavirus 1 [AfPV1])[1]

Discussion and Significance (OIE list, Zoonotic, notifiable): Few papillomaviruses have been molecularly identified in pinnipeds. Members of Papillomaviridae exhibit high host and tissue specificity, primarily infecting skin and mucosal surfaces. Infection can manifest in a variety of ways, from asymptomatic, to the formation of self-resolving papillary masses, to malignant epithelial cancers.[3] Associations between sporadic cases of Zalophus californianus papillomavirus (genus Dyonupapillomavirus) from California sea lions (*Zalophus californianus*) with squamous cell carcinoma have been reported.[2] In this case, the lesion was considered incidental and resolved over time.[1]

Deemed non-releasable, the animal was kept as part of a captive collection for 20 years and no neoplasia was detected at necropsy.

References

[1]Mifsud, J. C. O., Hall, J., Van Brussel, K., Rose, K., Parry, R., H., Holmes, E. C., Harvey, E. (2024). Identification of a novel papillomavirus from a New Zealand fur seal (*Arctocephalus forsteri*) with oral papilloma-like lesions. Viruses. In Press.

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[3]Syrjänen, S., 2018. Oral manifestations of human papillomavirus infections. Eur. J. Oral Sci. 126, 49-66.

CASE #6

Name: Graeme Knowles
Affiliation: Animal Health Laboratory, Mt Pleasant
Dept. of Natural Resources and Environment Tasmania
165 Westbury Rd Prospect Tasmania

Case Number: TARZ-15459.1(14/1400)

History with Clinical Signs and Signalment: This male, 5-year-old Tasmanian devil (*Sarcophilus harrisii*) was captured as part of a trapping survey in the Snug tiers, south of Hobart. The animal had significant weight loss. There was a large (greater than 70 mm across) red to dark brown ulcerated cutaneous mass extending across the left side of the face.

Gross Pathology: On necropsy, the ulcerated cutaneous mass extended into the oral cavity.

Microscopic Pathology: Skin. There is an ulcerated, densely cellular, non-encapsulated, expansile neoplasm with an infiltrative pattern of growth at the margins. The neoplasm consists of a pleomorphic population of cells. The cells vary from polygonal cells forming a solid pattern to spindle to plump spindle cells forming bundles, set in a fine eosinophilic fibrillar stroma. The polygonal cells have distinct cell borders, moderate amount of eosinophilic cytoplasm, oval nucleus coarse to vesicular chromatin pattern and single and occasionally multiple nucleoli. The spindle cells have distinct cell borders, moderate amount of eosinophilic cytoplasm, oval to elongated nucleus with stippled chromatin and single nucleolus. There are 5 mitotic figures per 2.37mm2 (10 x high powered fields; x 40 objective) and low to moderate numbers of apoptotic figures.

Ancillary Tests: Fresh tissue biopsies can be tested by PCR to detect DFT 1 or DFT2

Disease name: Devil Facial Tumour Disease, due to DFT2

Discussion and Significance (OIE list, Zoonotic, notifiable): Devil Facial Tumour Disease (DFTD) due to DFT2 cells was first found in 2014 amongst Tasmanian devil populations in the Snug tiers and near Cygnet, south of Hobart. This tumour type is transmissible.[1,2,3] Devil Facial Tumour Disease due to DFT1 has been found across almost all geographical regions of mainland Tasmania, but DFTD due to DFT2 has been restricted to the Snug Tiers and Cygnet region south of Hobart since it was first identified in 2014.

The cytogenetic analysis of DFT2, found on multiple Tasmanian devils, was key to supporting the transmissibility of the tumour and identifying that the tumour did not derive from the host. The DFT2 cells, compared to normal Tasmanian devil cells have additional material on chromosomes 1,2, and 4, a deletion involving chromosome 5 and monosomy for chromosome 6. Both X and Y sex chromosomes are present.[1] DFT2 similar to DFT 1, is of Schwann cell origin, but phenotypically is poorly differentiated and unlike DFT1 does not stain with periaxin. DFT1 and DFT2 can also be differentiated by PCR.[1]

Devil Facial Tumour Disease is a notifiable disease in most jurisdictions in Australia.

References

[1]Pye, R. J., D. Pemberton, C. Tovar, J. M. Tubio, K. A. Dun, S. Fox, J. Darby, D. Hayes, G. W. Knowles, A. Kreiss, H. V. Siddle, K. Swift, A. B. Lyons, E. P. Murchison and G. M. Woods (2016). "A second transmissible cancer in Tasmanian devils." Proc Natl Acad Sci U S A 113(2): 374-379.
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CASE #7

Name: Dr Heather Fenton
Affiliation: The Australian Registry of Wildlife Health
Taronga Zoo, Bradleys Head Road
Mosman NSW 2088

Case Number: TARZ 15297.1BB and PAS

History with Clinical Signs and Signalment: This free-ranging, adult, male, Eastern blue-tongue lizard (*Tiliqua scincoides*) presented to a veterinary clinic emaciated and minimally responsive to stimuli. Both eyes were scarred shut. The skin appeared purple in several places with lifting of the scales. The animal was euthanized, and the carcass was frozen.

Gross Pathology: Extensive regions of ulceration and proliferation with crusting were present affecting the head and neck, particularly the eye, ear, maxilla, and mandibles on the left side of the face and extending to the ventral neck. All limbs had multifocal ulcerative lesions that occupied approximately 20% of the limbs and were more severe proximal to the body. Multifocal, lesions of ulceration were present on the tail, flanks, and ventrum of the body. There were no subcutaneous or visceral fat stores. A small focal, tan nodule approximately 0.5-cm in width was present at the apex of the heart, that was raised and protrudes from the epicardium. The kidneys were diffusely pale. The gall bladder was distended, and the liver was diffusely dark green to black. The stomach was empty.

Microscopic Pathology: Multifocal to coalescing regions of erosion to ulceration were present with loss of epidermal architecture and replacement with loosely packed parakeratotic and orthokeratotic keratin infiltrated by polymorphonuclear cells, bacterial colonies and multiple round structures approximately 5-15 microns in diameter with a thick capsule (possible spores). In some sections, the spore-type structures appear pigmented. Deposits of purple granular material were present in some sections of skin (interpreted as mineral). Regions of skin adjacent to the ulcerated and eroded areas were occasionally hyperplastic. The inflammatory infiltrate extended within the dermis and subcutaneous tissue and was also associated with dense fibro-collagenous tissue.

The yeast-like structures stained with Periodic Acid Schiff (PAS) staining, in addition to fungal hyphae approximately 10 microns in diameter that were septate and have acute angle branching. In some areas, the hyphae were expanded with bulbous dilations. Occasional ovoid to rectangular arthrospores were present in some sections often within the keratinaceous debris.

No agents were visible with Ziehl Neelsen (ZN) staining. Bacterial colonies within the keratinized debris were mixed with large Gram-positive colonies of small coccobacilli and numerous larger Gram-positive cocci and coccobacilli within the debris.

The fungal hyphae described above were present within the foci of inflammation within the heart and the lung.

Ancillary Tests: A Diff-Quick stained post-mortem impression smear contained clumps of epithelial cells with occasional branching and segmented fungal hyphae approximately 5-10 microns in diameter amidst a pale blue background often with thin streams of blue material. Polymorphonuclear cells (likely heterophils) and foamy, vacuolated macrophages were also present throughout the smear.

Swabs from the oral cavity and the upper arm, as well as a skin sample were submitted to the Taronga Laboratory for fungal culture and two fungal isolates from the best two colonies were identified as *Nannizziopsis barbatae* by NSW Health.

Disease name: Formerly "yellow fungal disease" as a fungal disease of reptiles with fungal organisms originating in the *Chrysosporium anamorph* of *Nannizziopsis vriesii* [CANV] complex

Discussion and Significance (OIE list, Zoonotic, notifiable): The clinical history, gross, and microscopic findings are suggestive of a severe fungal infection. The severity of the lesions likely impacted this animals' ability to eat, see and ambulate, which likely explained the poor nutritional condition. There was evidence of systemic fungal spread to the heart and lungs in this case. The fungal agent isolated from multiple samples from this animal was identified as *Nannizziopsis barbatae*. This fungus is an emerging species associated with severe skin disease in captive and free-ranging Australian reptiles across the continent.[4] Although the taxonomy of the *Chrysosporium anamorph* of *Nannizziopsis vriesii* [CANV] complex is being re-evaluated, genera including *Nannizziopsis*, *Paranannizziopsis* and *Ophidiomyces* are known to cause potentially debilitating disease in reptiles with significance at the population-level.[1,2,3] Such infections may be associated with environmental or other causes of immunosuppression in addition to being significant biosecurity risks associated with international transport of reptiles. Fungal isolation includes incubation of the plates at 30 ^{o}C, as fungal pathogens tend to grow at lower temperatures that optimal mammalian body temperature.[4] The rectangular appearance of the arthrospores microscopically is characteristic of fungal organisms from this group.

References

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CASE #8

Name: Dr Heather Fenton
Affiliation: The Australian Registry of Wildlife Health
Taronga Zoo, Bradleys Head Road
Mosman NSW 2088

Case Number: TARZ-14372.1

History with Clinical Signs and Signalment: This adult, male, free-ranging green tree frog (*Litoria caerulea*) was found alive and dying in a pond. It appeared to have reddish-brown colouring on the back. The frog was found dead upon arrival at a veterinary clinic and was placed whole in formalin.

Microscopic Pathology: The epidermis was mildly thickened with hyperplasia and overlain with crusts of keratin with multifocal regions of erosion associated with small infiltrates of lymphocytes, plasma cells, and granulocytic leukocytes. Multiple fungal spores including flask-shaped thalli, multinucleated thalli, 10-micron zoosporangia and 2-3-micron basophilic zoospores were present within the epidermis also associated with bacterial colonies. Melanomacrophages are abundant within the dermis underlying adjacent intact epithelium.

Ancillary Tests: Could not be performed as the entire carcass was fixed in formalin, but PCR testing is available in Australia.

Disease name: chytridiomycosis

Discussion and Significance (OIE list, Zoonotic, notifiable): The microscopic lesions in the skin are consistent with the disease syndrome chytridiomycosis caused by the fungal agent *Batrachochytrium dendrobatidis* (Bd) or the chytrid fungus. The alterations in the skin barrier can be fatal to amphibians due to the functional importance of amphibian skin in maintaining appropriate ion balance and hydration.[1] Additional risk factors for mortality associated with chytrid fungus infection include environmental disturbances such as rapid ambient temperature changes and exposure to chemicals.[1,4,5] Hyperkeratosis, disrupted skin shedding, and pigmentation (that likely explained the reddish-brown colouration of the skin in this case) are common gross findings although in some cases the gross findings may be minimal. Many frogs can have subclinical infections with Bd without associated mortality with development of disease attributed to multifactorial relationships amongst the host, agent, and environment.[3] Global trade of amphibians has been implicated in assisting the spread of this pathogen worldwide.[5] As species susceptibility to the pathogen varies and disease can be associated with concurrent factors, it is considered a threat to amphibian conservation in many regions, with localised Australian amphibian extinctions attributed to Bd introduction.[1] This agent is notifiable to the World Organization for Animal Health (WOAH/OIE) within Chapter 8.1 of the aquatic code and is not considered to be zoonotic.[2]

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CASE #9

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Case Number: TARZ-15474

History with Clinical Signs and Signalment: Adult, female common ringtail possum (*Pseudocheirus peregrinus*) found at Silverleaves, Phillip Island, Victoria. Multiple deep ulcers were present on the limbs and tail.

Microscopic Pathology: Skin. There is sharply demarcated full thickness ulceration of the epidermis. The superficial and deep dermis underlying the ulcer and the adjacent normal epithelium is severely disrupted with focally extensive necrosis, with surrounding infiltration with neutrophils and fewer macrophages. On the ulcerated surface there are occasional aggregations of necrotic neutrophils and bacteria. A Ziehl-Neelsen stained section revealed large numbers of acid-fast bacilli in the dermis, often within the cytoplasm of macrophages and free within the necrotic debris.

Ancillary Tests: qPCR for *Mycobacterium ulcerans* was positive (performed at Victorian Infectious Diseases Reference Laboratory).

Disease name: Buruli Ulcer, Bairnsdale Ulcer, Daintree Ulcer

Discussion and Significance (OIE list, Zoonotic, notifiable): Skin disease in humans due to infection with *M. ulcerans* is notifiable to public health departments in many states. The bacterium can produce a cytotoxin mycolactone, which induces tissue necrosis and inhibits local immune responses. The condition is reported in many mammalian species, but is most common in humans, ringtail and brushtail possums. The lesions are often chronic deep cutaneous ulcers. Infection is endemic in certain areas of Victoria and far north Queensland. It has also been reported in the Northern Territory and the Batemans Bay area of New South Wales.[1] One hypothesis is that when the bacterium arrives in an area, possums are affected and can act as a maintenance host, with transmission to humans most likely occurring via arthropod vector (mosquitoes) or environmental exposure. A study at one endemic site demonstrated that *M. ulcerans* can be detected in faeces from possums with (82%) or without (14%) skin lesions.[2] The health department in Victoria is currently investigating qPCR for *M. ulcerans* in possum faeces, mosquitoes, and environmental samples for surveillance.

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CASE #10

Name: Dr Elizabeth Elsmo
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Case Number: R23-11041

History with Clinical Signs and Signalment: An approximately 3-week-old, male, free-ranging red fox (*Vulpes vulpes*) was found trapped in a flooded window well. After being recovered and admitted to a wildlife rehabilitation centre, it was weak, lethargic, and vocalising. The fox kit had a mildly elevated respiratory rate (80 bpm) with mildly increased effort and harsh lung sounds on auscultation. The kit was being transmitted to radiology to evaluate for evidence of trauma when it suddenly died.

Gross Pathology: A 400 gram, approximately 3-week-old, male red fox (*Vulpes vulpes*) is presented for necropsy. The fox is in fair nutritional condition, with a body condition score of 2/5. The lungs are diffusely light red and oedematous. The liver is mottled tan to maroon, with sharp margins. The stomach is empty. Intestinal contents and faeces appear normal.

Microscopic Pathology: Cerebral cortex. There are multifocal to coalescing regions of malacia affecting both the white and grey matter. Dense infiltrates of neutrophils and macrophages, as well as aggregates of karyorrhectic debris and increased numbers of glial cells are present in affected regions. There is scattered neuronal satellitosis, neuronal necrosis, and neuronophagia. Multifocal, fibrinoid vascular necrosis is present throughout the neuropil and Virchow-Robbins space is often filled by lymphocytes and plasma cells (lymphoplasmacytic perivascular cuffing). The leptomeninges are multifocally expanded by fibrin and infiltrates of lymphocytes, plasma cells, neutrophils, and macrophages.

Ancillary Tests: PCR testing of a brain sample was strongly positive (CT 7.6) for avian influenza virus using an avian influenza matrix real-time PCR assay. Further subtyping PCR at the National Veterinary Services Laboratory in Ames, IA, USA, confirmed this to be H5N1 highly pathogenic avian influenza, clade 2.3.4.4b.

Disease name: H5N1 highly pathogenic avian influenza virus

Discussion and Significance (OIE list, Zoonotic, notifiable): The H5Nx clade 2.3.4.4b of highly pathogenic avian influenza is a highly contagious viral infection, primarily of waterfowl and domestic poultry. This clade has caused significant economic impacts and population-level declines in affected species world-wide. Free-ranging waterfowl and shorebirds may be subclinical carriers or clinically infected. Infection in scavenging birds (i.e., raptors and vultures) and domestic poultry typically cause high mortality rates. Sporadic spill over into free-ranging and domestic terrestrial and marine mammals has been increasingly documented, typically causing neurological manifestations. This virus is potentially zoonotic with a range in clinical manifestations from subclinical illness to fatality in affected humans. Although not yet detected in mainland Antarctica, Australia or New Zealand, there are heightened concerns for poultry industries and wildlife in Oceania.

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Dr. Michelle Wille, University of Melbourne, The Peter Doherty Institute for Infection and Immunity. Avian Influenza Resources: https://www.michellewille.com/avian-influenza-resources/

CASE #11

Name: Dr Elizabeth Elsmo
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Case Number: R23-00826

History with Clinical Signs and Signalment: This adult female red fox (*Vulpes vulpes*) had been previously fitted with a GPS tracking collar and was found deceased. Post-mortem radiographs revealed no evidence of broken bones.

Gross Pathology: This fox was thin, with a body condition score of 2/5. The eyes are sunken, and mucous membranes were pale and tacky (consistent with dehydration). The skin over the pinnae, muzzle, limbs, and tail was diffusely thickened and scaly with areas of alopecia. The stomach contained abundant fur and small amounts of dark green liquid. The small and large intestine contain dark green to dark brown viscous digesta. No formed faeces were present in the colon. There were multiple segmental dark regions throughout both uterine horns, interpreted as placental scars.

Microscopic Pathology: Haired skin. There is diffuse acanthosis with marked orthokeratotic and parakeratotic hyperkeratosis. Within the stratum corneum are tunnels containing cross sections of 200-300 µm in diameter arthropod mites that have a spiny exoskeleton, jointed appendages, striated muscle, a hemocoel, and intestinal and reproductive organs. Also admixed within the stratum corneum are serocellular crusts, colonies of bacterial cocci, and ovoid to 'footprint'-shaped fungal yeast (likely *Malassezia* spp.).

Ancillary Tests: Sarcoptic mites identified in deep skin scrapings (cytology)

Disease name: Sarcoptic mange with secondary bacterial and fungal dermatitis

Discussion and Significance (OIE list, Zoonotic, notifiable): *Sarcoptes scabiei* is a highly contagious mite affecting many mammalian species world-wide, spread by direct contact or contaminated environments. Clinical signs of severe infestations typically present as intense pruritus, alopecia, and predisposes to secondary bacterial and fungal dermatitis. Zoonotic infections are possible, which are typically self-limiting and use of appropriate personal protective equipment (e.g., gloves) when handling infected animals. In Australia, several mammalian species have been documented with mange with population-level declines suspected to be associated with the disease in some species (e. g. bare-nosed [common] wombats, *Vombatus ursinus*).

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CASE #12

Name: Dr Anthony Chamings
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Case Number: M18-12354 (Grey-headed flying fox)

History with Clinical Signs and Signalment: An adult, female grey-headed flying fox (*Pteropus poliocephalus*) was found caught on a fence and unresponsive to stimulation and brought into care. On clinical exam, the flying fox was unable to hang by its feet, was lethargic, unresponsive, and exhibited muscular twitching. Over the course of the night the flying fox became progressively lethargic, and the paralysis worsened. The flying fox was euthanised the next day and submitted for a post-mortem examination.

Gross Pathology: No significant gross findings.

Microscopic Pathology:
Slide 3 Diencephalon, Hippocampus, cerebrum, and pineal gland
Multifocally, several blood vessels are surrounded by 1-2 cell thick cuffs of lymphocytes. Multifocally, adjacent to vessels with lymphocytic cuffs, there are multiple small areas of neuropil with gliosis and increased clear vacuolation (oedema) and fragmentation (malacia) and rare hypereosinophilic cellular debris containing pyknotic nuclei (necrosis). Multifocally, the meninges contain moderate numbers of lymphocytes and plasma cells.

Slide 4: Diencephalon (at level of the lateral geniculate bodies) and hippocampus
Multifocally within the diencephalon and hippocampus there are numerous blood vessels surrounded by 1-2 cell thick cuffs of lymphocytes and multifocally within the adjacent neuropil there are many foci containing moderate numbers of glial cells (gliosis). Multifocally, the meninges contain moderate numbers of lymphocytes and plasma cells.

Morphological diagnosis (from these sections and others of this case not shown here): Brain (Cerebrum, brainstem, and cerebellum); meningoencephalitis, lymphoplasmacytic, subacute active, multifocal, moderate with micromalacia, perivascular cuffing, and Wallerian degeneration

Ancillary Tests:
Australian Bat Lyssavirus (Pteropid) PCR: Positive,
Australian Bat Lyssavirus (Insectivorous) PCR: Negative,
Fluorescent antibody test for lyssavirus antigen: Positive,
Lyssavirus Immunohistochemistry: Positive,
Lyssavirus isolation: Detected

Disease name: Australian Bat Lyssavirus

Discussion and Significance (OIE list, Zoonotic, notifiable): Australian flying foxes and insectivorous bats are natural reservoirs for Australian bat lyssavirus (ABLV). Australian bat lyssavirus can infect humans and other mammals, and in infected individuals causes a fatal neurological disease (e.g.,

abnormal behaviour and paralysis) similar to rabies virus infection. Australian bat lyssavirus is a notifiable disease in all states and territories in Australia. Human exposure occurs via people being scratched or bitten by infected bats. People exposed should seek urgent medical attention to begin post-exposure prophylaxis. People working with bats should be vaccinated for rabies and wear appropriate personal protective equipment (PPE). A wide range of histological changes may be seen with ABLV infection in bats including, meningitis, perivascular cuffing, gliosis, Negri bodies (eosinophilic intracytoplasmic inclusions), neuronal necrosis, intracellular vacuolation and neuropil vacuolation. However, not all changes are seen in all cases and occasionally no histological lesions, or only sparse histological changes are observed in infected bats.

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CASE #13

Name: Anne Jordan
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Case Number: M22-14730

History with Clinical Signs and Signalment: Ten eastern grey kangaroos (*Macropus giganteus*) were found grazing in suburban Canberra. Two animals were found dead, and one was observed to be recumbent, and unable to rise with a poor body condition score. This animal was noted to have an otherwise normal neurological examination, dull mentation, and pale mucous membranes with mild icterus. It was subsequently euthanized and submitted to the Elizabeth MacArthur Agricultural Institute for post-mortem examination.

Gross Pathology:

- Little subcutaneous fat was observed, although the subcutaneous tissue present had a yellow tinge (icteric)
- Large volume of serosanguinous peritoneal fluid
- Liver with multiple foci of haemorrhage and prominent bile ducts filled with moderate numbers of small trematodes

Microscopic Pathology:

- Multifocal to coalescing foci of coagulative necrosis and fibrin thrombi surrounded by fibrosis
- Portal fibrosis and biliary hyperplasia
- Mixed inflammation (lymphocytes, plasma cells, macrophages, eosinophils, and neutrophils) in portal regions and associated with foci of necrosis
- Multifocal haemorrhage
- Extracellular and intracellular yellow-orange pigment often within Kupffer cells (possibly hemosiderin or bile)
- Multiple sections of adult trematodes with characteristic features (no body cavity, oral suckers, and hermaphroditic features) and eggs (thick yellow shell that is operculated).

Disease name: Liver fluke, hepatic fascioliasis, *Fasciola hepatica*

Discussion and Significance (OIE list, Zoonotic, notifiable)

- *Fasciola hepatica* (the sheep liver fluke), occurs commonly in macropods including eastern grey kangaroos, as well as other marsupials, grazing on wet pastures and sharing pastures with livestock.[1]
- Can result in significant hepatic pathology with heavy infection loads although subclinical infections are also possible.[1,3,4]
- Affected animals may exhibit weight loss, anorexia, depression, anaemia, and death.[1]
- There are concerns from an agricultural perspective that macropods could act as potential reservoirs for livestock infection and vectors for drug resistance.[3]
- Reports of larger numbers of impacted free-ranging animals tend to be associated with wet environmental conditions, likely due to environmental factors that influence the life cycle of the intermediate host, the *Lymnaea tomentose* snail.[1]
- Although infections in kangaroos have been reported as early as 1909 in Australia, it is considered to have been introduced associated with agricultural activities.[2]

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CASE #14

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Case Numbers: AS-23-2377 (slide 2 – H&E and MSB), AS-23-2450 (slide 3 – H&E and MSB)

History with Clinical Signs and Signalment: These cases were adult, male quokkas (*Setonix brachyurus*) from Wadjemup (Rottnest Island), Western Australia (WA). They were submitted as part of an investigation into an outbreak of respiratory disease progressing to mortality. Wadjemup is a 19km^2 island just off the coast of WA, 18km west of Fremantle and close to WA's capital city, Perth. It is a popular tourist destination with only ~300 permanent inhabitants, but ~780,000 annual visitors. Quokkas are small macropods (2.5-5kg, approximately the size of a cat) indigenous to the island; they are listed as vulnerable in accordance with the International Union for the Conservation of Nature criteria. On Wadjemup they are free-ranging and are not provisioned, instead grazing a range of both native and introduced grasses, shrubs, sedges, and leaves that grow on the island. Unfortunately, they have little fear of humans and commonly approach people who frequently feed them illegally. Over a 3-4 week period in June-July 2023, up to 10 quokkas were found either dead or moribund, with an estimated additional 30-50 live animals affected with respiratory signs, out of a total of an estimated 10,000-20,000 quokkas on the island. Affected animals were predominantly located in the northeastern region of the island. Both sexes were affected; all animals in the case-set were adults bar

one which was a juvenile. Clinical signs consistently shown by affected animals were wheezing through to overt dyspnoea, oculonasal discharge, sneezing, lethargy/depression, inappetence progressing to anorexia, poor body condition, moribund appearance, death. Some of the females had joeys.

Gross Pathology:

- Severe bilateral and diffuse pulmonary red-purple mottling (hyperaemia, hypostasis, haemorrhage), with a heavy, wet, and incompletely collapsed appearance (oedema).
- Bilateral multifocal subpleural and intraparenchymal areas of 'bubbly' gas pocket formation (emphysema).
- Bilateral multifocal flat red pleural lesions up to 5mm (petechial haemorrhage).
- Little to no adipose reserves, serous atrophy of intra-abdominal (incl. perirenal) adipose.
- Mildly increased peritoneal fluid varying from serosanguineous to fibrin-containing.

Microscopic Pathology:

AS-23-2377 (slide 2 – H&E and MSB): Four lung sections are present. Three show identical changes – alveolar septa are congested, and many alveoli contain pale eosinophilic fluid (modified transudate – protein-rich oedema) or flocculent to fibrillar/lacy material (fibrin) and variable numbers of macrophages and neutrophils. Rarely fibrin is seen compacting into more curvilinear structures starting to line the lumens of alveoli and alveolar ducts (putative early hyaline membrane formation). Multifocally, there is extensive compensatory expansion and coalescence of alveoli to form air-filled spaces (alveolar overinflation).

Many of the large bronchial airways are plugged with macrophages, neutrophils and lesser lymphocytes and plasma cells with sloughed bronchial epithelial cells (bronchitis). There is lifting of the bronchial epithelium with loss of epithelial cells (bronchial necrosis) and mixed inflammatory cells exocytosing through bronchial walls (necrotising bronchitis). In some bronchi these necrotic plugs are accompanied by large cloud-like colonies of coccobacilli which appear uniform in appearance. In the surrounding peribronchial pulmonary parenchyma there is influx of neutrophils accompanied by proliferating alveolar macrophages into alveolar spaces (bronchopneumonia with alveolar histiocytosis). Diffuse engorgement of alveolar wall capillaries and bronchial vessels with erythrocytes (congestion) with accumulation of fibrillar eosinophilic material expanding the interstitium (interstitial oedema). Approximately 70% of alveolar spaces are flooded with floccular eosinophilic material (fibrin rich oedema). There is multifocal compensatory distension of alveolar spaces (alveolar overinflation). No viral inclusion bodies are observed.

AS-23-2450 (slide 3 – H&E and MSB): Two lung sections are present. Changes are as described for the 3 slides described above for AS-23-2377; however fibrinous exudation is more prominent.

Morphologic Diagnosis: Severe, focally extensive, acute to subacute necrosuppurative bronchitis with bronchopneumonia and intralesional coccobacilli; severe, diffuse, acute fibrinous alveolitis and oedema (consistent with acute exudative phase of Diffuse Alveolar Damage).

Ancillary Tests:

- Routine bacteriology and mycology were performed on lung tissue:
    - AS-23-2377 - *Pseudomonas aeruginosa*, *Acinetobacter* spp and *Aeromonas hydrophila* isolated in mixed growth
    - AS-23-2450 – no growth
    - Both cases had no fungal growth
- Parasitology: AS-23-2377 – intestinal strongyle eggs detected on faecal floatation
- Pan-herpesvirus PCR on lung: negative both cases
- Influenza A PCR on lung: negative both cases
- COVID Rapid Antigen Test: negative both cases
- Electron microscopy on lung: no significant findings

- Whole genome sequencing on lung: no significant findings
- Gas chromatography and mass spectrometry (GCMS) toxin screening on gastrointestinal contents:
    - Cardiac glycosides not detected in either animal.
    - 3-methylindole detected in AS-23-2377 (but not AS-23-2450, repeat testing is ongoing).
- DNA barcoding for identification of plant material in gastrointestinal contents (AS-23-2377 only): samples were identified to genus level (*Ficus*) based on comparison with publicly available sequence information. Positive matches were made to several species of Ficus including *F. triloba* and *F. hirta* with high sequence similarity (98% or greater).
- Testing of gastrointestinal contents from more animals, as well as plasma, for 3-methylindole is ongoing.

Disease name: Suspected 3-methylindole toxicity, causing Diffuse Alveolar Damage (DAD)

Discussion and Significance: Acute fibrinous alveolitis and oedema is a common finding in these cases. The changes are consistent with the acute exudative phase of Diffuse Alveolar Damage (DAD).[1] Given the extent and severity of the changes, it is likely that the changes found were responsible for the described respiratory signs. These changes are aetiologically non-specific, and there are numerous infectious and non-infectious causes for DAD. The changes were quite acute, as there was negligible type II pneumocyte hyperplasia. It is likely that the bacterial necrosuppurative bronchitis and bronchopneumonia seen in 1 of 4 lung sections for AS-23-2377 represents secondary opportunistic infection. The bacteriology from the first case included the isolation of *Pseudomonas aeruginosa*, which is a known opportunistic pathogen and likely a secondary invader. *Ficus* spp. contain tryptophan within the stem and fruits.[2,3] In herbivores, L-tryptophan is metabolised in the rumen (or colon of non-ruminants) to 3-methylindole (3-MI), which is absorbed into the bloodstream. Cytochrome P450 enzymes in various locations (but especially the lungs) are largely responsible for the conversion of 3-MI to an electrophilic intermediate (3-methyleneindolenine) that alkylates cellular macromolecules, resulting in lipid peroxidation and membrane damage. This results in necrosis of non-ciliated and ciliated bronchiolar epithelial cells and type I pneumocytes, and transient endothelial cell swelling, resulting in DAD. In rodents, nasal respiratory (olfactory) epithelium is also targeted by 3-MI, due to the cytochrome P450-dependent biotransformation activity of the nasal mucosa and could have been responsible for the upper respiratory tract signs observed, although nasal cavity sections were not taken to assess histologically. This case bears similarity to other entities in domestic veterinary species in which 3-MI is believed to be a cause/contributory factor, particularly acute bovine pulmonary emphysema and oedema (ABPEE), which is also known as fog fever, atypical interstitial pneumonia, acute interstitial lung disease in feedlot cattle, and interstitial pneumonia in foals.[1] Interestingly, in ABPEE, nursing calves are not at risk and juveniles are less susceptible; the reasons for which are unknown. The *Ficus* spp. identified by DNA barcoding from the gastrointestinal tract are present on Wadjemup and have been on site for decades in areas where quokka frequent. Anecdotally, the quokkas do eat the leaves of this plant although they are not preferred and are not thought to comprise a large part of their diet, so it is not known what has changed to increase exposure although changing environmental conditions could have an impact on the availability and distribution of chemicals within the plant. Alternatively, an additional trigger for DAD cannot be completely ruled out. More testing is pending to hopefully confirm the presence of 3-MI in more samples from the case-set animals.

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CASE #15

Dr Jess Whinfield[1,2] & Dr Cheryl Sangster[3]
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Case Number: ARWH 13294.1

History with Clinical Signs and Signalment: An adult (>2-year-old), male platypus (*Ornithorhynchus anatinus*) was found deceased on private farmland in north-eastern New South Wales. The property owner regularly observed platypuses in a creek running through the property and had become concerned about one individual because it had been seen foraging during the day. A fresh deceased platypus was found one day and was suspected to be the same individual. This platypus carcass was taken to a local veterinary clinic where a gross necropsy was performed. Fixed tissues were sent to the Taronga Zoo for histopathology.

Gross Pathology: The gross necropsy was performed at a local veterinary clinic and no gross pathology was reported. Based upon post-mortem photos, the platypus was assessed as emaciated based on its Tail Volume Index (TVI). There was no other pathology grossly appreciable in the photos.

Microscopic Pathology:
Spleen: Expanding and effacing the red pulp and invading and effacing lymphoid nodules of the white pulp is an infiltrative and expansile population of cells. These cells are arranged in sheets within the pre-existing stroma. The cells are round with minimal cytoplasm and have large round to ovoid nuclei with vesicular chromatin and large, prominent nucleoli. There is moderate anisocytosis and anisokaryosis. The mitotic rate is 5 in 10 HPFs. Admixed with the neoplastic population are numerous plasma cells.

Heart: Multifocally and extensively, the ventricular myocardium is infiltrated by large numbers of similar cells as described above dissecting along fascial planes and between myocytes. Admixed with these cells are low numbers of lymphocytes and plasma cells.

Ancillary Tests: Immunohistochemistry for T cells (CD3) and B cells (CD79b) appear to work in platypus tissues, based on internal controls, but the cellular origin of this case could not be determined.

Disease name: Round cell sarcoma

Discussion and Significance: There are no peer-reviewed records of neoplasia in platypuses. As part of a retrospective review of platypus medical records (Whinfield et al., in prep.), three cases of neoplasia were found, which in all cases were diagnosed as round cell sarcomas. Two cases were identified in free-ranging platypuses, and one was from a captive individual (Sangster & Whinfield, in prep.). As of 2023, only a handful of cases of neoplasia in monotremes have been reported, including a splenic lymphoma and a soft tissue sarcoma, both in short-beaked echidnas (Tachyglossus aculeatus).[1,2]

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NOTES: