The value of post mortem examinations and histopathology in diagnosing diseases affecting and threatening Australian native animals

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Abstract

Most clinical signs of disease are non-specific and rarely reveal the full nature or causes of disease without extensive, expensive and sophisticated tests. For example, a possum showing signs of depression and ataxia (incoordination) may be affected by head trauma, systemic infection, local infection (eg, meningitis, pneumonia, stomatitis, hepatitis, cryptococossis, or middle ear infection), parasites (rat lungworm, possum lung worm, toxoplasmosis), toxins, neoplasia (tumours or cancer) or even nutritional deficiencies. Haematology, serum chemistry, radiology and ultrasonography can be expensive and often fail to indicate the correct diagnosis. By far the simplest, cheapest and most effective investigation is a thorough gross post-mortem examination followed by histopathology.

As for farm animals, the investigative approach for wild animals is based on "herd health" and a primary objective is to identify the presence of disease agents that may endanger the health and welfare of the population or community and not just the individual. Mostly, severely ill wild animals that are unlikely to be rehabilitated to the wild should be euthanised and whenever possible thoroughly investigated using gross necropsy and histopathology. If warranted, microbiological (such as bacterial or viral culture) and occasionally toxicological investigations may also be undertaken to identify specific agents. Otherwise carers and wildlife veterinarians can only continue to guess at what may be affecting local wildlife which may lead to inappropriate treatments and poor health and welfare outcomes for the animals. This presentation will describe the final diagnoses of common cases in Australian wildlife presented for treatment at the WAIF Wildlife Clinic that were only possible due to the use of gross necropsy and histopathology.

INTRODUCTION

A post-mortem examination (or necropsy or autopsy) is the examination of a carcase. In human pathology the primary purpose is to determine the cause of death, identify or characterise the extent of any disease present or to determine whether a particular medical or surgical treatment had been effective. Similar reasons probably apply to the post-mortem examinations of pet animals where the focus is still on the individual animal but for farm or wild animals the focus is on "herd health" and a primary objective is to identify the presence of disease agents that may endanger the health and welfare of the rest of the herd, population or community and not the individual. For

these reasons one or several animals may be killed to enable thorough investigations using gross necropsy, histopathological, microbiological and occasionally toxicological investigations to maximise the likelihood of identifying any agents of disease present most accurately and effectively.

In contrast to the impression given by CSI and similar television shows, a gross post mortem examination is not sophisticated or mystical. It is a thoughtful dismantling of the carcase to best observe the organs and tissues to identify any departures from normal and this can be performed quickly and is one of the most cost-effective diagnostic tests available. Unlike the heroes in novels and TV shows who make instant definitive diagnoses while glancing at exposed tissues, although necropsies are usually highly informative, gross tissue changes can be non-specific or even misleading so clients and carers should have realistic expectations.

Specimens for histopathology:

By far the commonest mistake is to place too few tissue specimens in formalin. This point cannot be over-emphasised. It is false economy to skimp on the number of tissues you put in formalin. Tissues keep indefinitely in formalin. Tissues not preserved in formalin are lost forever. If the cost of processing many tissues is problematic, submit 2 or 3; if the findings from these are inconclusive, send more.

Don't just sample the tissues that you think are significant. Clinical signs don't always reflect the primary site of the disease. For example, diarrhoea can be due to disease of the liver or kidneys, therefore to simply take specimens of gut from an animal with diarrhoea may miss the most important lesions. A reliable diagnosis can only be achieved with histopathology: gross findings at necropsy can be misleading so it is very important to sample a wide range of organs. Another pertinent example would be the common absence of identifiable gross pathology in the central nervous system despite devastating microscopic changes.

Generally specimens of the brain, heart, lung, liver, kidney, spleen and 3 or 4 levels of small intestine should always be taken. Additional tissues can be based on species' differences and the clinical history: the spinal cord should be sampled from an animal with spinal cord disease.

Quickly place tissue samples for histopathology into at least 10 times the volume (10:1) of 10% neutral buffered formalin to the volume of tissues to minimise autolysis. Tissues fixed for 24 hours or more can be re-packed in a smaller volume of fixative for posting to the laboratory. Tissue pieces must be less than 1 cm thick to ensure rapid penetration of fixative; only the brain is fixed whole. Label all containers and provide an accompanying letter detailing the history, gross findings, the animal's age, sex, breed, name, owner's name and your name and address.

An accurate, written objective record of the necropsy findings is essential to enable the histopathologists to determine. A digital photographic record of the case before death and all the tissues during necropsy is inexpensive and invaluable.

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WAIF Clinical Cases:

Case 1: A **female ringtail possum** was found huddled on the ground during the day in south Turramurra and was taken to the WAIF Wildlife Clinic where it was found to be hypothermic,

severely depressed with severe bilateral swelling of the face and bilateral conjunctivitis but the ears appeared to be unaffected. Initial treatment was warmth and parenteral antihistamine and corticosteroids but it died before further treatment could be instigated.





Necropsy findings:

Histopathology findings:

Diagnosis:

Case 2: Several **brushtail possums** presented blind with or without moderate to severe ataxia/paresis and disorientation. Otherwise they were generally in good condition and mostly calm:



Necropsy findings:

Histopathology findings:

Diagnosis:

Case 3: Several **brushtail possums** presented depressed with moderate to severe ataxia/paresis, one with hind limb paralysis:



This subadult BTP was about to be released when the carer realised it had become paralysed. It had been in care 6 weeks, looked very healthy, was eating well, mass 362 g increasing to 570 g 2 weeks later. Moved to outside aviary in preparation for release but didn't come out to eat over Easter so fed by hand: ravenous and ate well. Still not eating so examined: unable to move and very weak. Taken to another clinic where spinal injury was suspected and it was given a single steroid injection but deteriorated. Still ate well if food was put in its mouth.

Examination: mass 684 g, thin, poor musculature, unable to use hind limbs. Pinch test: no pain response by head but local withdrawal reflex strong. Same in both hind limbs and the tail: suspected complete spinal block.

Necropsy findings:

Histopathology findings:

Case 4: Several **tawny frogmouths** presented with severe leg paresis/paralysis or moribund:







Necropsy findings:
Histopathology findings:

Case 5: A **juvenile magpie** with a severe gape worm burden in its pharynx.



This image reveals the severity of the gape worm infestation and how badly they can affect the ability of the bird to eat and swallow let alone deprive it of nourishment and other resources.

Case 6: Adult magpie with severe limb dermatitis and osteomyelitis:

Necropsy findings:

Histopathology findings:

Case 7: A couple of **juvenile ringtail possums** with severe caecal bloat – unfortunately they both died but provided tissues for the very important research into ringtail and brushtail possum gastrointestinal bacterial microflora.

Necropsy findings:

Histopathology findings:

Diagnosis:

Case 8: A **mature brushtail possum** was treated with Betamox for severe dermatitis. It was eating and climbing well but the carer was concerned that it seemed to have problems breathing and may not be ready for release. When examined it had roughly symmetrical hair loss but despite its good condition and good reflexes it had severe breathing difficulties with harsh râles. It was put on twice daily Clavulox for 14 days and then to be returned for a recheck. When examined two weeks later its condition was the same and the respiratory problems had only improved marginally. Due to the severity of its condition it was euthanised.



Scaly dermatitis and alopecia (hair loss) in a mature BTP

Necropsy findings:

Histopathology findings:

Diagnosis:

Case 9: **Brushtail possum** in good condition with head injuries from a presumed dog attack. Other signs included a swollen head, pronounced snuffling with a dark watery discharge and it could only eat grapes.

Necropsy findings:
Histopathology findings:
Diagnosis:
Case 10: Juvenile male brushtail possum: had been examined at another veterinary clinic where it was diagnosed with a punctured lung because it was coughing, had obvious dyspnea (difficulty breathing) and blue mucous membranes indicating cyanosis likely due to compromised lung function. Examination revealed it was very thin, scruffy, with severe dyspnea (laboured open-mouth breathing) and mucous membranes that became blue when it struggled (only weakly). There were no râles (abnormal lung sounds) and the normal lung sounds were muffled and inaudible over most of the left side.
Necropsy findings:
Histopathology findings:
Diagnosis:
Case 11: Adult female brushtail possum had just been picked up lying on the front steps of a house. It was very thin with scruffy fur and alopecia (lost fur) on the rump, an old open wound on the left side of the mouth and was very weak although it tried to react aggressively when examined. Due to its very poor condition and poor prognosis it was euthanised.
Necropsy findings:
Histopathology findings:
Diagnosis:

Case 12: A **juvenile brushtail possum** was found lying on the ground in Sydney Botanical Gardens and taken to a local veterinary hospital. It was described as having an abnormal left eye and drank very poorly. On examination at the WAIF Clinic it was found to have an opaque left lens and exhibited ataxia when turned upside down and didn't move around when placed on the table. It was considered to be totally blind and euthanised. Samples were taken during a post-mortem examination and the cause of the blindness and neurological deficit will be investigated.



A blind and ataxic juvenile BTP

Necropsy findings:		
Histopathology findings:		

Case 13: Ringtail possum juvenile: hand reared since it was 68 g. It was very lethargic when it first came into care and still often takes fluids as if it is thirsty which may indicate renal damage. Became lethargic a week before presentation to the WAIF clinic and 5 days ago had dark green diarrhoea. The next day it displayed a clenched jaw with frothing and it was started on antibiotics. It stopped frothing the following day and began to pass normal faeces. It looked bright but did not eat well. A faecal examination was negative. It was discharged with fluids, probiotics and nystatin. It returned 3 weeks later with increased weight (350 to 410 g) and eating better but still not well. A watery mucoid green substance suspected to be caecal contents soiled the towel every night. Advice: pair with a tractable ringtail with normal mature GIT potentially to help seed its GIT with normal gut flora and to continue the milk formula and probiotic, adding a high energy/high protein supplement.

Represented 4 weeks later. Was given leaf "milkshakes" some with adult RTP faecal pellets over a few days. Appetite improved but still not normal. Passed normal and unformed pellets and looked bright. However, previous day was off-colour and this morning was gagging, its tongue was swoller and wet faecal-type matter was around the mouth.
Necropsy findings:
Histopathology findings:
Diagnosis:

Case 14: **Ringtail possum aged female**: one of several found dead in North Turramurra. Suspected poisoning so brought to clinic for investigation.

Necropsy findings:

Histopathology findings:

Diagnosis:

Case 15: **Sulphur crested cockatoos**: 3 fledglings, with varying amounts of pale grey on some head feathers. Rescued after their nesting tree was cut down. All eating well. One started to seizure the night before. Examination showed all in good condition and behaving normally except the one which demonstrated grand mal seizures every 5 minutes. It was euthanised. Blood samples from all 3 chicks were negative for Circovirus.



Sulphur crested cockatoo fledgling seizure

Necropsy findings:

Histopathology findings:

Diagnosis:

Case 16: **Ringtail possum**: adult female brought in as feeding during the day unconcerned about people. Assessed as blind (no menace response and walked into walls). Euthanised.

Necropsy findings:

Histopathology findings:

Diagnosis:
Case 17: Tawny frogmouth : adult presented with neurological signs, weak on left side and although it could perch it would fall to one side. Mentation, pupillary reflexes were normal but the range of movement of both wings and the strength of its legs were reduced. No improvement in a week so it was euthanised.
Necropsy findings:
Histopathology findings:
Diagnosis: