5.4. Pathology

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Abstract

From September 2005 to May 2007 officers from Department of Environment and Conservation and staff from Australian Wildlife Conservancy, submitted woylies found dead in monitored wild populations in southwestern Australia and Karakamia Wildlife Sanctuary (Chidlow, WA), respectively, to the Murdoch University pathology department. Moribund animals were sent to Perth Zoo. Field data associated with each animal submitted, for example site description, weather, evidence of predators (scats, tracks, etc), and state of the remains, was recorded by the submitters.

Thirty one woylies were submitted for necropsy at Murdoch University. Thirteen were males, 16 female and two were unknown (because only limited body parts were found in the field). All animals submitted were adults. One female adult had a pouch-young.

Veterinary pathologists from School of Veterinary and Biomedical Sciences, Murdoch University conducted all necropsies. The causes of death included;

- skeletal fractures/ marked haemorrhage (7) (mostly suspected road accidents)
- skeletal fractures/haemorrhage with puncture (bite) wounds (6) (predation)
- cardiomyopathy / skeletal muscle myopathy (2)

In 11 cases the cause of death could not be determined grossly or by histopathology. All but one of the animals, whose cause of death was unknown, was in good body condition. For this reason, in conjunction with the unremarkable gross and microscopic findings, an acute process rather than chronic disease was the likely cause for the unknown mortalities.

Four animals presented moribund with marked focal dermal lesions and one had septic arthritis. Culture results indicated these were isolated cases of non-transmissible diseases.

5.4.1. Introduction

There has been a sudden decline in the woylie population in southwestern Australia within the past 2 years. To investigate cause for woylie decline Department of Environment and Conservation coordinated (through the Woylie Conservation Research Project) three areas of research; predation, resources and disease.

DEC requested Murdoch University and Perth Zoo to investigate disease factors that could contribute to the decline. Methods used to investigate disease factors included necropsy of animals found dead as well as assessment of live captured animals (blood taken for clinical pathology, faecal analysis for endoparasites, external parasites).

5.4.2. Methods

From September 2005 to May 2007 officers from Department of Environment and Conservation and staff from Australian Wildlife Conservancy, submitted woylies found dead in monitored wild populations in southwestern Australia and Karakamia Wildlife Sanctuary (Chidlow, WA), respectively, to the Murdoch University pathology department. Moribund animals were sent to Perth Zoo, and examined clinically. They were all euthanized for humane reasons and submitted to Murdoch University for necropsy. Field data including, for example site description, weather, evidence of predators (scats, tracks, etc), and state of the remains, was recorded by the submitters.

Staff veterinary pathologists from the School of veterinary and biomedical science, Murdoch University, conducted all necropsies. Histopathology (using haematoxylin and eosin stains) was conducted on tissues from all animals submitted (unless the carcase was markedly degenerate). If indicated (e.g. gross changes indicating inflammatory or necrotic processes) tissues were sent for bacterial culture. Intestinal contents were taken for parasitological assay (McMaster method). Stomach contents were taken to assess diet. Ethanol fixed heart, skeletal muscle and brain was set aside for PCR assay for *Toxoplasma* (Bretagne, 1993).

In addition, multiple organ samples (including liver, spleen, kidney, heart, and lung) were frozen and oropharyngeal swabs held frozen in virus transport media. These were kept for possible future molecular assays or virus culture to further investigate the cause of death where gross and microscopic findings were unremarkable.

Swabs of bite wounds (on the collar), from one of the animals which had evidence of trauma and bite wounds at necropsy, were sent for PCR analysis to identify the predator species. The assay used mtDNA primers for the control region and cytochrome b gene for species identification (P Spencer personal communications).

5.4.3. Results

5.4.3.1. Gender and age

Thirty one woylies were submitted for necropsy at Murdoch University. Thirteen were males, 16 female and two were unknown (because only limited body parts were found in the field). All animals submitted were adults. One female adult had a pouch-young.

5.4.3.2. Causes of death and morbidity

The cause of death was concluded most commonly from gross assessment.

Trauma was the most common cause of death identified. No other findings were evident in these animals to indicate intercurrent or chronic disease. That is, there were no factors found that could have predisposed to predation or other forms of trauma.

Table 5.4.1. Overall assessment of cause of death and morbidity of woylies submitted for necropsy.

Number of animals	Cause of death	Cause of morbidity
7	Skeletal fractures/ marked haemorrhage	
6	Skeletal fractures/haemorrhage with puncture (bite) wounds	
2	Cardiomyopathy / myopathy	
11	Unknown	
4		Chronic focal dermatitis
1		Chronic septic arthritis
31	Total	

The majority of animals with skeletal fractures/ marked haemorrhage were found near the road suggesting the cause of death was road accidents.

The one case of cardiomyopathy showed microscopic changes strongly suggestive of exertional cardiomyopathy. This occurs following excessive adrenaline release and marked sudden demand on cardiac function. The skeletal myopathy was unusual with regenerative changes evident only in the tongue and oesophagus.

In 11 cases the cause of death could not be determined grossly or by histopathology. All but one of the animals, whose cause of death was unknown, was in good body condition. For this reason, in conjunction with the unremarkable gross and microscopic findings, an acute process rather than chronic disease was the likely cause for the unknown mortalities.

Four animals presented moribund with marked focal dermal lesions and one had septic arthritis. The bacteria isolated from the skin lesions included *Bacillus cereus*, *Staphylococcus* spp, *E. coli*

and *Pasteurella* spp. Mixed bacterial growth was isolated from the septic arthritis lesion. The culture results indicated these were isolated cases which were due non-transmissible.

The location where the animal was found was recorded and this has been tabled below against the cause of death or morbidity

Table 5.4.2. Location and cause of death and morbidity of woylies submitted for necropsy.

	Cause of death				Cause of morbidity	
Site	Skeletal fractures/ marked haemorrhage	Predation	Cardiomyopathy / myopathy	Unknown	Chronic septic arthritis	Focal dermatitis / osteomyelitis
Keninup	2	1				
Balban	1					
Karakamia	1			4	1	4
Chariup			1			
Manjimup	1					
Batalling	1					
Dryandra		3		3		
Tutanning	1	2		4		
Wellard			1			

5.4.3.3. Parasitology

Intestinal content or faeces were not collected from all submitted animals. If the animal remains were markedly degenerate samples were not taken.

Table 5.4.3. Nematode or eggs identified from woylies submitted for necropsy.

	Strongyle eggs	Lungworm larvae	Adult protostrongyle woylie	Unknown or degenerate larvae	Adult parastrongylus bettongia	No nematode or eggs seen
Number of animals	5	1	1	2	1	13

5.4.3.4. *Toxoplasma* PCR

To date, only three woylie tissue samples were tested for *Toxoplasma* DNA. This was using PCR outlined in (Bretagne *et al*., 1993). All samples were negative.

5.4.3.5. PCR analysis of bite wound swabs

Swabs of the bites on the tracking collar of one animal with multiple bite wounds and haemorrhage into the chest (supporting predation as the cause of death) were submitted for PCR but the results were unrewarding and the species of the predator could not be confirmed.

5.4.4. Discussion

The cause of death was determined in 15 out of 26 cases submitted for necropsy (5 other animals were euthanized due to chronic dermal lesions or septic arthritis). Most commonly the cause of death was concluded based on gross findings. Trauma was the most common cause of death. No other findings were evident in these animals to indicate intercurrent or chronic disease. That is, there were no factors found grossly or microscopically that could have predisposed to predation.

In 11 cases the cause of death could not be determined grossly or by histopathology. All but one of the animals, whose cause of death was unknown, was in good body condition. For this reason, in conjunction with the unremarkable gross and microscopic findings, an acute process rather than chronic disease was the likely cause for the unknown mortalities. Surveys of sudden death (unexpected death in apparently healthy animals) of companion animals and equines (including necropsy and histopathological assessment) often have a significant proportion of cases of unknown causes of death varying from 30% to 60% (Brown, 1988; Knowles, 2006).

Bloods taken from collared animals which were later found dead will be analysed and the haematology assessed to see if this will further clarify a cause of death in those cases of unknown sudden death.

Two groups of differential diagnoses for sudden death in woylies with unremarkable gross and microscopic findings may be considered. These are causes

a) which relate to individual deaths for example defects in cardiac conductivity, snake bite, lightning strike, hypothermia

b) infectious / toxic causes affecting a common population e.g. botulism, acute plant toxicoses (e.g. plants containing cardiac glycosides, fluoroacetate compounds).

The interests of this study are in the latter group of differential diagnoses i.e. diseases affecting a population group. Botulism is uncommonly reported in marsupials. Testing of fresh feed or serology (blood sample) is required to assess for botulinum toxin. These samples were not available. Plant toxicology relating to native marsupials is limited. Assessment of fresh feed is required. Of course an unknown infectious or toxic agent cannot be excluded as the cause of the sudden death. Serology will be undertaken from trapped woylies for orbivirus, herpesvirus and flavivirus. Please note that chorioretinitis (orbivirus infection in kangaroos), epithelial or hepatic necrosis (herpesvirus) or encephalitis (flavivirus) which are commonly microscopic findings associated with these viruses were not noted in the cases examined.

The case of cardiomyopathy showed microscopic changes strongly suggestive of adrenaline induced cardiomyopathy. This occurs following excessive adrenaline release (due to severe acute stress) and marked sudden demand on cardiac function. The second case of myopathy displayed chronic skeletal muscle regeneration secondary to muscle degeneration but only in selective sites (tongue and oesophagus). A cause could only be speculated based on extrapolation from ruminants. In ruminants causes of myopathy include selenium and vitamin E deficiency, ingestion of toxins such as gossypol, *Cassia* spp or prolonged intensive anaerobic exertion (Van Vleet and Valentine, 2007).

Those woylies which had intestinal parasites were in reasonable body condition and there were no significant gross or microscopic pathological lesions in the intestinal tract. For these reasons the endoparasites identified were incidental findings and did not contribute to the cause of death or morbidity.

5.4.5. Future work

The haematology of collared animals, which were later found dead (and necropsied), will be used to further investigate possible predisposing factors to morbidity and death. Serological surveys of blood from trapped woylies will be undertaken in the next month at UWA. Serological assays for orbivirus, herpesvirus and flavivirus will be undertaken. Based upon serological results, PCR analysis or virus culture of stored samples may be considered on those animals that had inconclusive necropsy findings. Assessment of stomach contents from dead woylies for seeds of known toxic plants will also be considered.

5.4.6. Conclusion

This was a small survey of dead and moribund woylies (31 animals submitted in total). There was no predominant cause evident for the death of woylies submitted. Road accidents and predation were causes of death. DEC staff, having knowledge of the historic records of woylie populations, will interpret the importance of these factors. Further analysis of haematological data (for collared / tracked woylies) is pending. This will be particularly helpful for those cases of sudden death of unknown cause. Further molecular assays based on serological surveys to assess for exposure of the woylie population to orbivirus, herpesvirus and flavivirus infection will be considered for the stored tissues from the animals with unresolved causes of death.

5.4.7. References

Bretagne, S., J. M. Costa, *et al.* (1993) "Detection of *Toxoplasma* gondii by competitive DNA amplification of bronchoalveolar lavage samples" J Infect Dis 168(6): 1585-8

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