

## PATHOLOGY OF MACROPODS

Philip W. Ladds  
Graduate Research College, Southern Cross University  
P.O. Box 157, Lismore, NSW 2480

Revised by  
Ian K. Barker  
Canadian Cooperative Wildlife Health Centre, Ontario/Nunavut Region  
Dept. of Pathobiology, Ontario Veterinary College,  
University of Guelph, Guelph, ON, Canada N1G 2W1

Most information in these notes is covered more fully in 'Pathology of Australian Native Wildlife' (Ladds, 2009), in which references to cited literature, other than those added in revision, are given.

Readers are referred to the publications below for information on the systematics, biology, anatomy, physiology, captive management and medicine of macropods, which may aid in interpretation of disease processes encountered by the pathologist.

**Armati, P.J., C.R. Dickman & I.D. Hume (eds). 2006. Marsupials. Cambridge University Press, Cambridge. Pp 373.**

**Coulsen, G. & M. Eldridge (eds). 2010. Macropods. The Biology of Kangaroos, Wallabies and Rat-kangaroos. CSIRO Publishing, Collingwood. Pp 408.**

**Jackson, S. 2003. Australian Mammals. Biology and Captive Management. CSIRO Publishing, Collingwood. Pp 524.**

**Ladds, P. 2009. Pathology of Australian Native Wildlife. CSIRO Publishing, Collingwood. Pp 640.**

**Tyndale-Biscoe, H. 2005. Life of Marsupials. CSIRO Publishing, Collingwood. Pp 464.**

**Vogelnest, L., & R. Woods. 2008. Medicine of Australian Mammals. CSIRO Publishing, Collingwood. Pp 686.**

### 1. VIRAL DISEASES

#### Herpesviruses

**Alphaherpesviruses** (Macropod Herpesvirus-1 and -2) have been associated with disease in a number of macropod species, virtually always in captive animals, although there is serologic evidence of wide exposure to herpesviruses in free-ranging macropods. These agents are capable of latency, have been detected in the trigeminal ganglion, and may recrudesce in immunocompromised animals.

In general, **clinical signs** include respiratory rales, conjunctivitis, discharge from the eyes and nostril, incoordination, vesicles 2 to 3 mm in diameter in the labial and gingival mucosa and ano-genital region, depression, anorexia, and death.

**Gross lesions** can include the mucocutaneous vesicles and ulcers seen clinically; pulmonary congestion and edema; pinpoint red foci in the lungs; and pale 1-5mm diameter foci scattered throughout the liver. In parma wallabies a mucoid tracheitis was observed.

**Microscopic changes** included lymphoid depletion and apparent lymphocytolysis in germinal centres in the spleen, and multifocal necrosis in the liver. Eosinophilic to basophilic intranuclear inclusions are scattered in hepatocytes around necrotic foci. In experimental infections in parma wallabies, inclusions were not seen in liver, but necrosis with mineralisation was a feature.

**Gammaherpesvirus** infection (Macropod Herpesvirus-3) recently has been reported in a captive collection of eastern grey kangaroos in North America and in a free-ranging animal in Victoria. In the captive collection the virus was detected in a population with a high prevalence of mammary tumours, in individuals with clinical ulcerative cloacitis. The Victorian case was from a population, including animals relocated subsequent to the bushfires of 2009, in which a small outbreak of mortality was

noted, though none were necropsied. Animals were observed with ataxia, oculo-nasal discharge, lethargy and recumbency. Mucosal lesions were not noted in the single animal examined clinically. However, a gammaherpesvirus similar to that described in the captive animals was detected by PCR on swabs, but not isolated. While the association of a gammaherpesvirus with clinical disease in these cases is not proof of causality, it indicates that it should be suspected in grey kangaroos with ulcerative mucocutaneous lesions and/or respiratory disease.

**Diagnosis** of herpesviruses is suggested by the pattern of gross and microscopic lesions and confirmed by virus isolation, PCR, or presumptively, by electron microscopy on tissue sections.

**Wilcox, R.S., P. Vaz, N.P. Ficorilli et al. 2011. Gammaherpesvirus infection in a free-ranging eastern grey kangaroo (*Macropus giganteus*). Australian Veterinary Journal 89: 55-57.**

#### **'Cytomegalovirus'**

Enlarged epithelial cells containing prominent eosinophilic intranuclear inclusions have been described as an incidental finding in the renal collecting tubules of Eastern Bettongs. The agent involved has not been confirmed, but ultrastructurally, it is consistent with an adenovirus, rather than a herpesvirus, since no enveloped particles were observed in the cytoplasm of affected cells.

#### **Poxvirus and papillomavirus**

The lesions caused by these viruses represent separate disease entities. However, some reports of clinically similar proliferative skin lesions in macropods and several other native Australian species are ambiguous.

**Papillomaviruses associated with typical warts** have been described among marsupials only in brushtailed possums and woylies, although papillomavirus genome has been detected on the skin of clinically normal koalas and from an eastern grey kangaroo, among the seven macropods surveyed. The **lesions on the woylie** were comprised of multiple projecting papillary folds, with marked orthokeratotic and parakeratotic hyperkeratosis. The thick stratum spinosum contained typical koilocytes, and immunohistochemistry and in situ hybridization revealed papillomavirus signal in nuclei, although intranuclear inclusions were not described.

**Lesions compatible with poxvirus infection** in the skin of macropods (quokka, tammar wallabies, agile wallabies, swamp wallabies, wallaroos, red kangaroos, eastern and western grey kangaroos), are described in all ages, but mostly young animals. They may be solitary or multiple, and occur in skin on any part of the body, such as the tail, head or limbs. Two types of lesion, varying from a few mm to about 5 cm in diameter, are described. Whether these lesion types are distinctly different or whether there is a single entity, which in some animals may produce multiple lesions resembling human molluscum contagiosum, is unresolved. This latter type, seen infrequently, is an umbilicated, firm to hard papule that is non-tender. More common are proliferative, wart-like lesions that are irregular, have a roughened, hyperkeratotic surface, and become hairless and darker as they enlarge.

The microscopic appearance depends on the type of lesion and stage of development or regression. In those resembling molluscum contagiosum, hyperkeratosis and accumulation of trapped keratin in deeper locations may give the lesion a cup-shaped appearance at low magnification. In the papillomatous lesion there is marked acanthosis and hyperkeratosis, the thickened epidermis being directly continuous with normal epidermis. The more superficial cells are enlarged, often vacuolated, and may contain intracytoplasmic, eosinophilic inclusions up to approximately 45 µm in diameter which displace the nucleus. The amount of dermal inflammation associated with these lesions varies. There may be necrosis of the superficial part of the lesion, with a marked local neutrophilic infiltrate, and a lymphoplasmacytic infiltrate may be evident in the dermal papillae and subjacent dermis. While typical poxviruses have been demonstrated using electron microscopy, neither isolation nor molecular characterisation seems to have been accomplished.

**Bennett, M.D., A. Reiss, H. Stevens et al. 2010. The first complete papillomavirus genome characterised from a marsupial host: a novel isolate from *Bettongia penicillata*. Journal of Virology 84: 5448–5453.**

## **Arboviruses**

Serology and virus isolation have confirmed that infections with arthropod-borne viruses are common in native mammals in Australia, but with the exception of Wallal, and perhaps Warrego and Eubenagee viruses, although infection and viraemia in native species may amplify the viruses, they are not associated with overt illness or demonstrable lesions.

Two significant systemic diseases of macropods caused by orbivirus infections are recognised, epidemic blindness (choroid blindness, chorioretinitis of kangaroos), and a sudden death syndrome in tammar wallabies.

**Epidemic blindness:** Blindness, associated with infection by a midge-transmitted orbivirus of the Wallal serogroup, and possibly Warrego virus, has been observed in western grey kangaroos, and less often in eastern greys, red kangaroos and euros.

Gross lesions directly attributable to the viral infection are not reported.

Microscopically, in both spontaneous and experimental cases there is chorioretinitis with uveitis and often an accompanying non-suppurative encephalitis or meningoencephalitis. In some animals, however, there is encephalitis with no ocular lesions. In acute lesions chorioretinitis is severe and necrotising, with proteinaceous exudate, and neutrophils and gitter cells in the reaction. It may be segmental, or so extensive that little normal retinal tissue remains. In older lesions, chronic retinitis may be associated with segmental retinal atrophy, perhaps with little inflammation, and Wallerian degeneration of the optic nerve and optic tracts in the brain. Significant changes are not evident in other (non-ocular) tissues.

**Sudden death syndrome in tammar wallabies:** Sudden deaths associated with an orbivirus of the Eubenagee serogroup have been described in captive tammar wallabies. Mortality rates can be high, and most animals died without showing signs.

Grossly, consistent findings included pulmonary congestion, subcutaneous oedema of the hindlimbs and inguinal region, and mottling of the liver. Additionally, in about 30% of animals, there was extensive haemorrhage in many areas including fascia and muscle of the hindlimbs, the inguinal and dorsal cervical regions, ventral thorax and perirenal area.

Microscopically, congestion and haemorrhage, especially in the liver, were confirmed and in some there was acute periacinar hepatic necrosis. The gross and microscopic findings are consistent with endothelial damage and disseminated intravascular coagulation, pathogenetic mechanisms common to orbiviral diseases such as Bluetongue and Epizootic haemorrhagic disease.

**Rose, K.A., P.D. Kirkland, R.J. Davis et al. 2012. Epizootics of sudden death in tammar wallabies (*Macropus eugenii*) associated with an orbivirus infection. Australian Veterinary Journal. In press.**

## **Encephalomyocarditis virus**

Encephalomyocarditis (EMC) virus, a picornavirus, has been identified by immunohistochemistry in the myocardium of a quokka, and was also diagnosed or suspected as the cause of myocarditis and death in captive macropods.

Gross findings appear to be similar regardless of species, and include focal or diffuse pallor of the myocardium, perhaps mostly of the ventricles, and pulmonary congestion.

Microscopically, necrotising non-suppurative myocarditis is a consistent lesion, with accompanying fibrosis in some, presumably more chronic, cases.

## **Other viruses**

**Foot and mouth disease (FMD) virus:** Following experimental infection with the FMD virus, lesions – consisting of vesicles on the tongue and foot pads – were noted in red kangaroos and (Matschiei's) tree kangaroos (*Dendrolagus matschiei*). It was concluded unlikely that these species would play a significant role in the spread of FMD.

A recent report, however, described FMD in an eastern grey kangaroo captive in a zoological garden in India. Signs were those of sloughing of footpads, pain sufficient to prevent the animal from standing, and death two days after the onset of signs. Microscopically, focal myocardial necrosis and myocarditis were observed. Culture and typing by ELISA confirmed the presence of type O FMD virus.

## 2. BACTERIAL INFECTIONS

Pathological changes observed in animals with bacterial infections, especially acute infections, are chiefly those of septicaemia, are seldom specific, and probably for this reason are rarely reported. Fortunately, as is the case with most injurious agents, in sub-acute and chronic disease with persistent infection or exposure, host response usually becomes increasingly obvious, and the type of response may be highly specific or even pathognomonic.

### (a) Gram-positive bacteria

#### Staphylococci

Included here are infections with *Staphylococcus aureus*, *S. epidermidis*, *S. xylosus* and *S. capitis*. Whereas *S. aureus* often appears to be a primary pathogen the other staphylococci are normally non-pathogenic or opportunistic.

Although most Australian native terrestrial mammals are susceptible to staphylococcal infection, some species differences have been noted; macropod joeys seem to have innate resistance to *S. aureus* infection.

**Gross findings** may be those of peracute septicaemia with widespread petechial haemorrhage. Localising lesions vary with the duration of infection – acute lesions being fluctuating, and oedematous or suppurative, with later lesions being firmer due to abscess formation and fibrosis, and sometimes granuloma formation (botryomycosis).

**Microscopic findings** may be confounded by concurrent infection with other bacteria, especially if the lesion is superficial or cutaneous. Although the inflammatory response in staphylococcal infections seems mostly to be exudative and suppurative, the presence in some cases of so-called 'club colonies' surrounded by a pyogranulomatous response gives the lesion some specificity which is helpful in diagnosis of the botryomycotic form of staphylococcosis. This change has been observed in the koala, bilby, and echidna, and may presumably occur in macropods.

**Diagnosis** at necropsy is based on culture of localising lesions, or of several parenchymatous organs (liver, spleen, lung) in the case of suspected septicaemia. Histologically staphylococci are recognised as large Gram (+) cocci, sometimes forming clusters.

#### Streptococci

Most reports of disease in Australian native land mammals associated with streptococcal infection incriminate  $\beta$ -haemolytic streptococci but in some reports no species identification is given.

Infection with both haemolytic and non-haemolytic streptococci occurs frequently and may be secondary to, or concurrent with, other microbial infections such as in candidiasis in young hand-reared kangaroos, or with *Fusobacterium necrophorum* and other bacteria in 'lumpy jaw' necrobacillosis. In such cases the precise contribution of streptococci to illness cannot be assessed. Infection of wounds is commonly due to  $\beta$ -haemolytic streptococci as well as other bacteria such as *Staphylococcus aureus* and *Corynebacterium* spp. On the feet and lower limbs these may be evident as suppurative pododermatitis with tenosynovitis, and purulent to caseous inflammation and necrosis of the draining lymph nodes.

*Streptococcus equisimilis*, usually a human pathogen, was isolated from a fatal osteomyelitis lesion mid-shaft in the radius and ulna of a mature eastern grey kangaroo. Necropsy revealed a small sinus filled with creamy yellow pus.

**Diagnosis** is based on culture of localising or suspected primary lesions, or of parenchymatous organs in suspected septicæmias. Streptococci can be recognised in tissue as small Gram (+) cocci, often arranged in pairs or sometimes small clusters.

### **Arcanobacterium**

*Arcanobacterium pyogenes* is a pyogenic, Gram (+), pleomorphic, cocco-bacillary organism that can cause a wide variety of nonspecific suppurative lesions involving various visceral organs. As with staphylococci and streptococci, *Arcanobacterium pyogenes* frequently is isolated from inflamed lesions due to trauma sustained in capture of wallabies and several other native mammals. Footpads are especially involved with resultant suppurative pododermatitis, tenosynovitis and lymphadenitis. In untreated animals, septicæmia and embolic pneumonia may follow.

### **Clostridia**

**Tetanus**, resulting from infection of wounds with *Clostridium tetani*, is described as common in marsupials but opinion on its significance in these species is divided. It is reported in several macropod species. Signs, which may be of sudden onset and rapid progression, include protrusion of the nictitating membrane, dilation of nostrils, and marked tetanic contraction of muscles, especially those of the head, neck, thorax, forelimbs and tail. Mastication becomes 'impossible', presumably due in large measure to so-called 'lock jaw', and pulmonary oedema – as evidenced by frothing at the nostrils and dyspnoea – develops, and death occurs within several days.

Because pathological changes in tetanus are minimal, diagnosis must be based on clinical signs. Tearing of muscles associated with severe terminal spasms has however been observed, and lesions providing anaerobic conditions conducive to germination of *C. tetani*, may also be found. Such lesions have included a dog bite, and compound fractures of metatarsal bones.

**Enterotoxaemia** is reported in young kangaroos. Necropsy revealed petechiation on serous surfaces, and much translucent, straw-coloured fluid in body cavities. Within the small intestine there were petechiae and much 'custard-like' ingesta, a smear of which, when examined after staining with polychrome methylene blue, was seen to contain moderate numbers of bacterial rods consistent with *Clostridium perfringens*.

**Tyzzler's Disease**, caused by *Clostridium piliforme*, has been reported in diverse native mammals including an eastern grey kangaroo, and a male wallaby of unrecorded species. Most reported cases of Tyzzler's disease involved captive juveniles in which disease appeared suddenly and progressed quickly, with death occurring in one to several days.

Gross findings were chiefly observed in the liver, which was enlarged, congested and mottled, perhaps with an accentuated lobular pattern and scattered haemorrhages, and multiple pale, grey-white foci up to 3 mm in diameter throughout the parenchyma. Unlike in other diseases producing focal hepatic necrosis, including herpesviruses, and Gram-negative septicæmias, in which there is splenic enlargement or focal necrosis, in Tyzzler's disease, the spleen is normal or perhaps shrunken. Typhlocolitis, often seen in eutherian mammals with Tyzzler's disease, seems not to have been reported in macropods.

Histologically, in the liver the significant change in all cases was multifocal necrosis. The causal bacilli were usually discernible at the edge of lesions as faintly basophilic rods in sections stained with Haematoxylin and Eosin. They do not take Gram stain, but with silver (GMS) staining were seen clearly as skeins of distinct, often beaded rods.

### **Erysipelas**

Perhaps because clinical signs and pathological findings of *Erysipelothrix* sp. infection in native fauna lack specificity, erysipelas in these species seems under-reported. There is an early report of *E. rhusiopathiae* causing 'raised red' lesions on the inner aspect of the hindlimb of two kangaroos, and erysipelas has also been diagnosed in a captive wallaby with septicæmia manifested by muco-haemorrhagic enteritis, epicardial petechiation and splenomegaly.

Microscopically, *E. rhusiopathiae* is distinguished as one of the only two Gram (+) coccobacilli likely to be encountered in tissue (the other is *Listeria*). Microcolonies may be found in capillaries and small

venules throughout the body, associated with microvascular thrombosis, which probably explains the haemorrhage which often is evident grossly in some tissues, such as epicardium. *E. rhusiopathiae* is zoonotic, usually causing localised digital cellulitis, termed 'erysipeloid', and, rarely, valvular myocarditis or septicaemia. Confusingly, 'erysipelas' in people is a dermal cellulitis caused by *Streptococcus*.

### ***Actinomyces* and *Nocardia***

These organisms are difficult to culture, and may occur in mixed infections with *Fusobacterium*. Diagnosis often is based on their appearance in tissue sections, where both produce pyogranulomatous inflammation. *Actinomyces* appears filamentous, and unequivocally Gram (+), while *Nocardia* is variably Gram (+), forming beaded filaments, and stains weakly acid fast by a modified Ziehl-Neelsen method. Both are environmental organisms which may be ingested or found in the mouth. They cause opportunistic infections, often gaining entry to deeper tissues via a breach in the skin or oral mucosa, where especially *Actinomyces* may be found in lesions of 'lumpy jaw'. Actinomycosis, apparently unassociated with jaw lesions, was diagnosed as the cause of abdominal abscesses in a captive kangaroo.

Nocardiosis in macropods appears especially to involve the lungs, perhaps through inhalation of contaminated material, with acute respiratory embarrassment being observed clinically. Necropsy may reveal pyogranulomas, multi-focal caseous abscessation or involvement of an entire lobe, with adhesions between lobes, and serosanguinous exudate in the pleural cavity. Microscopy reveals the typical appearance of the organism.

### ***Listeria***

Listeriosis due to infection with *Listeria monocytogenes* has been diagnosed in a red-necked wallaby and a rufous bettong. It is an environmental opportunist, and the host likely is immunocompromised. A constant gross finding was the presence of a variable number of pale foci of necrosis, pinpoint to approximately 2 mm in diameter throughout the liver. Microscopically, these foci were confirmed as necrotic areas which were infiltrated by neutrophils or mixed inflammatory cells including histiocytes, and with scattered Gram (+) coccobacilli, usually intracellular, at the periphery of the lesion.

## **(b) Gram-Negative bacteria**

With the exception of *Fusobacterium*, *Bacteroides* and *Bartonella*, infections with the Gram (-) bacteria described here are diagnosed based on a compatible suite of lesions, associated with isolation of the agent from tissues with lesions, and from two or more parenchymatous organs, if septicaemia is suspected.

### ***Fusobacterium necrophorum* infection (Necrobacillosis)**

Necrobacillosis, a necrotising inflammation involving bone and or soft tissues, may be acute or chronic, and although caused primarily by *Fusobacterium necrophorum*, it may be exacerbated by concurrent infection with a variety of other microorganisms. *Fusobacterium* is an environmental opportunist, present on the ground, and in ingesta. To colonise it requires a breach in the epithelium of the skin, gingiva, oral cavity or upper gastrointestinal tract, after which its necrotizing toxins and leukocidins promote soft tissue necrosis with relatively little peripheral inflammation.

Necrobacillosis, especially involving the jaw (so called jaw disease or 'lumpy jaw'), is a common disease in captive macropods. All macropod species appear to be susceptible.

Perhaps surprisingly, animals may appear relatively normal until shortly before death – which occurs a few days to several weeks after the initial appearance of signs. The lesions probably start at the alveolar gingiva or at some breach in the gingival mucosa elsewhere, which may be related to trauma, dental progression, or sharp components in the diet.

Typically, gross lesions are primarily necrotising and involve mucous membranes, but in some very acute cases there is diffuse cellulitis of subcutaneous tissues of the face with minimal oral involvement.

As lesions develop there is characteristically an inner necrotic zone and an outer fibrous zone with both layers enclosing pockets of pus or caseous material. Usually ulceration or fistulation is apparent.

The pus is yellow-green and has a foul odour. Bony changes in more chronic cases, as anticipated from clinical observations, include formation of sequestra – usually involving the dental alveoli – and sub-periosteal bone formation with increasing deformity, especially of the mandible. Extension of infection to contiguous organs such as parotid salivary glands, nasal turbinates or brain may occur.

Visceral lesions can include multiple nodules containing yellow caseous material over the gastric serosa and throughout the liver, and necrotising foci in the lung, liver and stomach, perhaps related to swallowing or inhaling exudate containing massive numbers of bacteria, which set up local lesions in the lungs or stomach, the latter metastasizing via the portal flow to the liver.

Microscopically, the essential lesion in soft tissues is focal necrosis associated with masses of tangled Gram (-) filaments, which may be seen by careful observation in H&E sections. Other opportunistic bacteria, such as *Bacteroides* and *Actinomyces* also may be present. Necrosis is at first coagulative but later there is total loss of tissue structure. The bacteria may be only at the periphery, or be present throughout necrotic areas. Necrotic foci are in turn surrounded by leukocytes, predominantly mononuclear cells, many of which are degenerating. Beyond the leukocytes is a variable zone of fibroplasia. Necrotising lesions within bone are comparable to those in soft tissues and may be sharply demarcated from normal bone. Necrotic bone may become lytic and lost, and occasionally the entire distal mandible will slough, bilaterally. Marked reactive osteogenesis also may occur, contributing to mandibulo-maxillo-facial distortion.

**Diagnosis** is based on the characteristic suite of lesions, and recognition of the organism in lesion smears or tissue section. It is anaerobic, and rarely is isolation attempted.

### ***Bacteroides***

*Bacteroides* is a gastrointestinal anaerobe, most commonly recognised as an opportunistic contributing pathogen in 'lumpy jaw' caused primarily by *Fusobacterium* infection. However, *Bacteroides* sp. was isolated from lung of a long-footed potoroo with fibrinous pleuropneumonia. *Bacteroides* produces clumps or microcolonies of small basophilic organisms visible in H&E sections at the periphery of areas of coagulative-liquefactive necrosis in tissue.

### ***Salmonella***

Most *Salmonella* isolates from mammals are *Salmonella enterica*, ssp. *enterica*; the species and subspecies names are usually dropped, and the organism is designated by serotype in normal font, but initially capitalised (eg. *Salmonella enterica enterica* Typhimurium is expressed as *Salmonella* Typhimurium).

In Australia, many serotypes of *Salmonella* have been isolated from wildlife, especially macropods, but few reports relate particular isolates to clinical findings and even fewer describe lesions. *Salmonella* Typhimurium appears to be the main serotype associated with disease.

Clinical illness, death, and or lesions due to *Salmonella* infection are reported in many species of macropod, perhaps with the red kangaroo being over-represented and therefore possibly more susceptible – although removal from its normally arid environment may have heightened susceptibility. Importantly, salmonellosis appears not to be reported in free-ranging macropods, even though they may frequently be carriers of infection.

Death may occur without signs being observed. When present, signs are those of enteritis and septicaemia. Diarrhoea is the most consistent sign. In orphaned macropod joeys, loose faeces may contain streaks of blood sometimes progressing to profuse bloody diarrhoea.

Gross findings in fatal salmonellosis may include marked petechial and ecchymotic haemorrhages, pulmonary oedema, splenomegaly, and variably normal to fibrinohaemorrhagic intestinal mucosa, with luminal diarrhoea, fibrin, blood and casts, depending on the type of mucosal lesion.

In two acute cases in immature western grey kangaroos, in which gross lesions were not seen, there was microscopic focal hepatic necrosis, focal inflammation and necrosis of the muscularis of the small intestine, and suppurative bronchopneumonia. Focal hepatic necrosis with cholangitis was also seen in a pretty-faced wallaby with salmonellosis.

### ***Escherichia coli***

*E. coli* has been shown to be a significant pathogen, together with other Gram-negative bacteria, in macropod joeys – causing enteritis with bloody diarrhoea, peritonitis, pneumonia and septicaemia. Gross findings are those of a 'dull' peritoneum with occasional fibrin strands but no pus present. Extensive parts of the lung may be reddened and oedematous or consolidated, but not necrotic.

### ***Pseudomonas***

*Pseudomonas* spp. are opportunistic bacteria which occasionally cause serious disease in mammals either as primary pathogens or in association with other microorganisms.

*Pseudomonas* spp. are frequently isolated from cases of 'lumpy jaw' and have been recognised as pathogens in association with *Fusobacterium necrophorum* and other gram-negative bacteria in 'stressed' pademelons with pneumonia.

*Pseudomonas* infection appears to be more prevalent and serious in macropod joeys than adults; the organism often is isolated from the faeces of orphan joeys. Other than peritonitis and the frequent presence of pneumonia, lesions of septicaemia in *Pseudomonas* spp. infection in these joeys are not reported. But oedema, congestion, excess fluid in body cavities, and petechial haemorrhage, might be expected.

### ***Bordetella bronchiseptica***

There are several reports of illness or mortality of wallabies associated with *B. bronchiseptica* infection.

At necropsy, marked congestion and consolidation of the lungs were apparent. Microscopically, neutrophils were within bronchi and alveoli in affected lung, while in more normal areas, there was an increased prevalence of alveolar macrophages. Subacute pneumonia and pleurisy were diagnosed.

### ***Pasteurella***

Pasteurellosis, caused mostly by infection with *Pasteurella multocida*, is described in a variety of Australian native mammals including kangaroos and potoroos.

No doubt because of its presence in the upper respiratory tract of healthy animals, respiratory lesions in *P. multocida* infection are often found. Gross lesions in macropods have included bronchopneumonia, fibrinous pneumonia or pleuropneumonia. Other gross lesions in fatal pasteurellosis in kangaroos include haemorrhages in the gastrointestinal tract and lymph nodes.

In captive potoroos that died of pasteurellosis the lungs were diffusely red and sank partly in 10% formalin. Severe, lobular, acute-subacute pneumonia that was sometimes necrotising and, in more chronic cases, walled-off abscesses, were noted.

### ***Yersinia***

In common with several other gram-negative bacteria, *Yersinia pseudotuberculosis* may be isolated from the faeces of apparently healthy wild birds and animals. Under stressful conditions, however, fatal disease may occur.

Yersiniosis is described in a variety of Australian native mammals including kangaroos, and the red-necked wallaby. Yersiniosis is a zoonotic disease. *Yersinia* infects via the faecal-oral route, so lesions are distributed accordingly.

Macroscopically, widespread serosal petechiation and enteritis with congestion, haemorrhage and perhaps fibrinohaemorrhagic ulceration may be observed in acute cases. But *Y. pseudotuberculosis* often produces a characteristic picture of enteritis with necrosis of intestinal lymphoid tissue, mesenteric lymphadenitis, and hepatic and splenic areas of necrosis, seen as white foci, sometimes accompanied by consolidating embolic pneumonia.

Histologically, in affected portions of the intestine there is mucosal necrosis with mats of bacteria or aggregates of coccobacilli forming microcolonies visible in H&E sections, along the mucosal surface and within necrotic debris, often in gut-associated lymphoid tissue. In the more chronic cases, the discrete pale foci seen at necropsy consist of foci of necrosis infiltrated with neutrophils and macrophages, the latter predominating around lesions of some standing.

### **Proteus**

*Proteus* spp. are frequently isolated from both healthy and diseased Australian native mammals. They seldom are incriminated as primary pathogens. *Proteus* spp. are frequent isolates from the faeces of apparently normal animals as well as from those with disease.

*Proteus vulgaris* and *P. mirabilis* were isolated together with other bacteria from intestinal content of kangaroos with fatal stonyloidosis, and from orphaned macropod joeys. *Proteus morgagnii* and *P. rettgeri* have also been isolated from cases of pneumonia, and *P. rettgeri* has been isolated from a case of peritonitis in a joey. Lesions are comparable to those in coliform infections.

### **Burkholderia**

*Burkholderia pseudomallei* is an opportunistic soil- and water-borne organism that causes melioidosis, which, in people, has a relatively high fatality rate. Personal protective measures should be followed rigorously if necropsying an animal which may have died of melioidosis. Although serological surveys indicate that exposure to *Burkholderia pseudomallei* is widespread in Australian native mammals, especially in northern Australia, infection rates are not high, and there are few reports of overt disease, or descriptions of associated lesions.

Gross findings in melioidosis in Australian native mammals appear in general to be similar to those seen in domestic species. The pattern of lesions suggests infection via the intestinal tract, with dissemination via the portal blood to the liver and subsequent septicaemia. In a tree kangaroo that died of melioidosis after an observed illness of only a few days, multiple foci of necrosis were present in an enlarged, discoloured liver. In wallabies, similar pale foci up to about 1 mm in diameter, which were sometimes confluent, were throughout the liver and spleen, but pus was not observed.

### **Chromobacterium**

*Chromobacterium violaceum*, a bacterium found in soil and water in the tropics, which occasionally causes serious illness in man and other species, was reported as the cause of septicaemia in a young captive agile wallaby. At necropsy the lungs did not deflate, were oedematous, and contained multiple haemorrhages up to approximately 4 mm in diameter. Microscopically, pulmonary hyperaemia, oedema and haemorrhage were confirmed, and fluid was present in alveoli and bronchioles. Bacteria were prevalent throughout the lung, being most abundant in areas of exudation and haemorrhage.

### **Morganella**

*Morganella morgani* was isolated, in mixed culture with *Bacteroides* spp. and a  $\beta$ -haemolytic *Streptococcus* from lung lesions of a common wallaroo with hypertrophic osteopathy.

### **Bartonella**

*Bartonella australis*, a facultative intracellular Gram (-) bacterium has recently been isolated from blood of eastern grey kangaroos in central coastal Queensland, and a number of other *Bartonella* species have been recognised in fleas parasitising marsupials, including several macropods. No description of any associated illness or lesions is available, and pathogenicity of these organisms in macropods or humans is unknown. In view of possible human health implications, and the syndrome of anaemia and deaths of eastern grey kangaroos associated with presence of an unidentified haemoparasite (see under protozoa), the need for further investigation of *B. australis* is indicated.

**Kaewmongkol, G., S. Kaewmongkol, L.M., McInnes et al. 2011. Genetic characterization of flea-derived *Bartonella* species from native animals in Australia suggests host–parasite co-evolution. *Infection, Genetics and Evolution* 11: 1868-1872.**

## **(c) Spirochaetes**

### **Leptospira**

Review of the literature on leptospirosis is complicated by the detailed nomenclature based traditionally on serological cross-testing, and, by the relative lack of concordance of the results of such testing with genospecies determinations determined by DNA analysis of these organisms – which morphologically are identical.

A number of surveys involving serology, and sometimes bacteriological culture of mostly healthy animals, have investigated the presence and prevalence of leptospiral infection in Australian native mammals. Antibodies against leptospire have been demonstrated in a wide diversity of species including various macropods but no associated lesions appear to have been reported in macropods. *Leptospira interrogans* may be shed in urine of carrier animals, and is zoonotic.

#### **(d) Mycobacteria**

In many Australian native mammals, the presence of acid-fast bacilli (AFB) within lesions has been shown by culture or by other means, such as PCR, to reflect infection with *Mycobacterium* spp., including *M. bovis*, *M. tuberculosis*, *M. ulcerans*, *M. avium paratuberculosis*, *M. terrae*, *M. asiaticum*, *M. chelonae*, *M. fortuitum*, *M. chitae*, *M. smegmatis*, *M. avium* and *M. intracellulare*.

Mycobacteriosis is reported in a variety of macropods, emphasising the ubiquitous presence of mainly opportunistic mycobacteria in the environment. In macropods in South Australia, gross lesions suggestive of Johne's Disease (JD), microscopic changes consistent with JD, and positive tissue culture for *M. avium* subspecies *paratuberculosis*, indicated a likely epidemiological link between JD in domestic ruminants and macropods.

A review of cases in macropods published up to 1986 revealed that lesions of mycobacteriosis were seen most frequently in spleen and lymph node and less often encountered in bone, the respiratory system, then liver, in that order. In subsequent reports, involvement of the skin and subcutis is described more often, perhaps indicating caution in earlier reports of incriminating AFB as the primary pathogen in view of their ubiquitous nature and frequent identification in contaminated superficial lesions – together with other likely pathogens. The typical lesion in macropods varies from small miliary foci to massive nodules with an internal consistency varying from purulent to caseous. 'Grittiness' due to mineralisation occurs sometimes but is not a common finding.

Microscopically in macropods, lesions of mycobacteriosis consist typically of central areas of necrosis surrounded by histiocytes and giant cells. Peripheral to these cells are aggregates of lymphocytes, fewer plasma cells and polymorphonuclear neutrophils, and fibroplasia. Giant cells are usually present but their prevalence is variable. Mineralisation is not a constant feature. Acid fast bacteria tend to be plentiful in caseous areas but variable and, often rare, in more proliferative granulomatous lesions.

#### **(e) Rickettsia**

Major interest in rickettsioses is their zoonotic potential and the role native species may serve as reservoirs of infection. Although serological surveys have demonstrated that infections with *Coxiella burnetii* and *Rickettsia* spp. occur in a wide range of Australian native marsupials and rodents, clinical disease is not apparent and lesions seem not to have been described. In fatal experimental infection in one of two rufous rat kangaroos with *C. burnetii*, however, there was apparent splenomegaly and focal necrosis in the liver.

**Potter, A.S., M.J. Banazis, R. Yang et al. 2011. Prevalence of *Coxiella burnetii* in western grey kangaroos (*Macropus fuliginosus*) in Western Australia. Journal of Wildlife Diseases 47: 821-828.**

### **3. MYCOTIC DISEASES**

#### **Aspergillosis**

Respiratory aspergillosis has been observed in captive macropods including wallabies and wallaroos, but lesions were not described. Also *Aspergillus* sp. was isolated from and demonstrated within oesophageal tissues of a captive kangaroo.

#### **Candidiasis**

Candidiasis in Australian native mammals is caused mostly by *Candida albicans* but other *Candida* spp. such as *C. catenulata*, *C. tropicalis* and *C. krusei* are sometimes involved. Macropodids with candidiasis include kangaroos, wallabies, the wallaroo, and the musky rat-kangaroo.

Candidiasis may be localised in the gastrointestinal tract, cloaca, or skin, or be systemic, so signs will vary accordingly. Clinical examination of affected animals may reveal pale plaques or ulcers in the oral cavity on the tongue, gums and lips – which may be swollen, and pharyngitis of varying severity. Skin, especially around the mouth, may also be affected.

In addition to those lesions seen clinically, gross changes in kangaroos included linear white encrustations adherent to the folds of oesophageal epithelium, and distension of the non-glandular stomach with curd-like material much of which was adherent to the epithelium. In systemic candidiasis in a juvenile kangaroo, multiple, discrete necrotic but firm foci up to 5 mm in diameter were present throughout the liver. These foci were light brown and depressed below the surface.

Microscopically, although *Candida* preferentially invades the superficial layers of squamous epithelium, in severe infections deeper layers of such epithelium, as well as mucosa and contiguous tissues may be invaded.

Liver lesions only were reported in one eastern grey kangaroo with systemic candidiasis, the causal organism, *C. tropicalis*, presumably having metastasised from the gut, lung or some other location during a protracted illness. Foci of necrosis in the liver were associated with many fungal elements, including blastospores, pseudohyphae and true mycelia.

### **Cryptococcosis**

Cryptococcosis is most commonly caused by two species, *Cryptococcus neoformans* (consisting of two varieties - *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans*), and *C.gattii* (formerly *C. neoformans* var. *gattii* or *C. bacillospor*).

Cryptococcosis is reported in several diverse species of macropodids. Cryptococcal infection that is clinically apparent seems often to be opportunistic.

Signs in a captive long-nosed potoroo initially presented with weight loss, weakness, muscle atrophy and inappetence, included a subsequent head-tilt with circling, recumbency and visual deficits. Lesions of cryptococcosis described in both Gilbert's and long-nosed potoroos were those of meningoencephalitis, with optic neuritis also being seen in the latter species.

### **Other deep or systemic mycoses**

***Pneumocystis carinii*** was diagnosed by microscopic examination of impression smears in a captive red kangaroo with lesions of gastritis from which *Candida albicans* was isolated. In Giemsa-stained smears eight intracystic uni-nucleated bodies were seen. These bodies were light blue and their nuclei red but the cyst wall remained unstained. The cysts measured 5 to 7  $\mu$ m in diameter. With methenamine silver staining the cysts were black and thick walled. No description of lesions was given.

**Coccidioidomycosis** is reported in wallaroos and a red kangaroo held in zoos overseas. Necropsy of the kangaroo, which apparently had shown no signs of respiratory disease, revealed a solitary, firm, discrete mineralised lung lesion measuring 11 x 8 mm, with a fibrous wall measuring 1 to 2 mm in thickness. Microscopically, a granulomatous response was associated with spherules and free endospores characteristic of *Coccidioides*.

### **Dermatomycoses**

Ringworm in wildlife species is of particular interest because of its zoonotic aspects and the occurrence of clinical disease in carers and others handling wildlife or products thereof.

In several extensive surveys of Australian native mammals various fungi, including some potentially pathogenic species, notably *Trichophyton* sp. and *Microsporum* sp. were isolated, but in no case was isolation associated with clinical disease. Typical ringworm is overwhelmingly a disease of captive animals; severe dermatophytosis caused by *Microsporum persicolor* was however observed in a red kangaroo shot in the wild.

Ringworm has been reported in kangaroos, wallaroos, and wallabies. *M. canis*, or more often *T. mentagrophytes*, have been involved.

In kangaroos ringworm may present as an area of alopecia with minor reddening of the skin and 'little else'. Two forms of the disease are recognised in macropod joeys. In the 'classic' form there are discrete, sometimes multiple areas of alopecia with no erythema. In the more severe, generalised, form quite large areas may be involved and the skin is roughened and thickened with associated alopecia. There is a tendency for scabs to form. In young animals infection of the ears may lead to necrosis and sloughing of parts of the ear.

Microscopic findings in ringworm appear to be similar across species. Skin changes are most prominent in hair follicles, which show ortho- and parakeratotic hyperkeratosis, acanthosis and dilatation. Follicular lumens may be full of neutrophils, fungal spores and hyphae – the latter being seen to infiltrate keratin. Some of the follicles rupture, resulting in focal pyogranulomatous dermal inflammation. In orphan joeys it was noted that scales of hyperkeratotic epidermis were also invaded by hyphae and spores.

Identification of the causal fungus requires other tests such as culture, but presumably, as in domestic animals, *Microsporum* infections are generally characterised by a mosaic of polygonal arthrospores in an ectothrix pattern whereas in *Trichophyton* infections there are chains of arthrospores in both endo- and ectothrix patterns.

### **Mycotoxicosis**

Mycotoxicosis due to sporodesmin was suspected in a two year-old eastern grey kangaroo. Death occurred after a 3 to 4 week illness. Grossly the liver was small, firm and pale, and microscopically there was a severe, diffuse cholangiohepatopathy with marked bile duct hyperplasia. At the time of the incident, sporodesmin toxicity was reported in cattle in the area.

## **4. PROTISTAN DISEASES**

### **Toxoplasmosis**

*Toxoplasma* infection, confirmed by pathological examination or serology is described in many Australian native species, and it is suggested that vertical transmission may occur in macropods. Caution must be placed on some earlier reports, which may have involved related organisms such as *Neospora*, *Hammondia* or other tissue cyst-forming protists.

*Toxoplasma* infection is reported in most macropod species – in which the disease is often fulminating – with minimal signs occurring before ultimate prostration and death.

**Gross findings:** Often no changes indicative of toxoplasmosis are observed at necropsy, and if present, such changes are quite variable in severity. In a detailed correlative gross-microscopic study of toxoplasmosis, necropsy findings (or lack of them) in macropods with fatal toxoplasmosis included: no visible lesions, pulmonary congestion or consolidation, gastrointestinal reddening and ulceration, myocardial haemorrhages and pale streaks, splenomegaly, cerebral malacia, adrenal enlargement and reddening, pancreatic swelling, and lymphadenomegaly.

In this comparative study, diffuse or patchy pulmonary congestion, oedema and consolidation were common in affected macropods, and white foci were present in lung parenchyma. Myocardial haemorrhages were usually petechial and often interspersed with white streaks. Affected hearts were enlarged due to retention of blood in the ventricles and atria. Gastro-intestinal changes were confined primarily to the stomach and small intestine, and included ulceration and frequent patchy reddening, with white foci. In the pancreas, swelling due to oedema was often accompanied by irregularly scattered white foci of varying size. Malacic changes in the brain mostly involved the cerebral cortices.

**Microscopic findings:** Since gross lesions often are lacking or are inconsistent in animals with toxoplasmosis, diagnosis usually depends on observing the characteristic microscopic changes – associated in most cases with the free or encysted tachyzoites of *T. gondii*. Toxoplasmosis should be suspected in a case with any combination of non-suppurative meningoencephalitis, non-suppurative myocarditis, interstitial pneumonitis and focal hepatic and/or splenic necrosis. In spite of the great variability across species in the microscopic changes seen, it seems remarkable that brain,

myocardium and lung are so consistently involved, and that the predominant changes are necrosis and some form of non-suppurative inflammation. Whereas multi-organ, multi-focal necrosis associated with many organisms is a feature of acute fulminating toxoplasmosis in susceptible animals, more often there is encephalitis characterised by microglial nodules with some peri-vascular cuffing, myocarditis, and interstitial pneumonia. In such cases organisms are sometimes numerous but otherwise may be difficult to find, and usually are not associated directly with foci of necrosis, or with inflammatory foci in the brain. Diagnosis in such cases is facilitated by use of specific immunohistochemical staining.

**Parameswaran, N., R.M. O'Handley, M.E. Grigg et al. 2009. Vertical transmission of *Toxoplasma gondii* in Australian marsupials. *Parasitology* 136: 939-944.**

**Parameswaran, N., R.C.A Thompson, N. Sundar et al. 2010. Nonarchetypal Type II-like and atypical strains of *Toxoplasma gondii* infecting marsupials of Australia. *International Journal of Parasitology* 40: 635-640.**

**Portas, T.J. 2010. Toxoplasmosis in macropodids: a review. *Journal of Zoo and Wildlife Medicine*. 41: 1-6.**

### **Sarcocystosis**

Sarcocysts have been recognised in a variety of Australian native mammals including macropods. *Sarcocystis* infections in marsupials generally appear to be innocuous, but as the early stages of infection are pathogenic in some domestic animals, it is possible that disease may occur in marsupials – serving as intermediate hosts – in special circumstances.

In the overwhelming number of reports, sarcocysts were found only on microscopic examination of muscle. However, in some species they may be up to several mm in length, and thus be detected macroscopically.

### **Other Cyst-forming Apicomplexan Protists**

***Besnoitia*** spp. are characterised by production of usually multilocular intracytoplasmic cysts (meronts containing merozoites) in markedly hypertrophic host connective tissue cells, often in superficial locations on the body (dermis, ocular sclera, etc.). They are presumed to have a heteroxenous life cycle similar to *Sarcocystis*, involving a prey species in which the meronts are found, and a predator species in which sexual development occurs. However, a definitive host is not known for a number of the species of *Besnoitia* found in large mammals, in which mechanical transmission by arthropods is suspected.

In some species the hypertrophic host cells are sufficiently large (~1 mm) to be visible to the naked eye in superficial locations, or at necropsy.

A single case of besnoitiosis has been described in a captive western grey kangaroo in Western Australia, and an allusion was made to besnoitiosis associated with epistaxis in wild kangaroos. Further information is required on the prevalence and distribution of this organism before work on its biology and significance can be determined. No *Besnoitia* are zoonotic, and most are probably fairly host-specific.

**Anon. 2010. *Besnoitia*. Western Australian Animal Health Report, July 2010, Pp. 9.**

**Olias, P., B. Schade & H. Mehlhorn. 2011. Molecular pathology, taxonomy and epidemiology of *Besnoitia* species (Protozoa: Sarcocystidae). *Infection, Genetics and Evolution* 11: 1564-1576.**

***Neospora caninum*** meronts were associated with myocarditis in a captive parma wallaby that died in a zoo in Europe. Immunohistochemistry and/or molecular techniques would be required to differentiate *Neospora* from *Toxoplasma* in such cases. While *Neospora caninum* is established in wild dog populations in Australia, and causes disease in cattle, the possibility of a sylvatic cycle involving native wildlife is speculative.

**Cronstedt-Fell, A., B. Richter, T. Voracek et al. 2011. Neosporosis in a captive parma wallaby (*Macropus parma*). *Journal of Comparative Pathology* (June 2011 - Epub ahead of print).**

**King, J.S., D.J. Jenkins, J.T. Ellis et al. 2011. Implications of wild dog ecology on the sylvatic cycle of *Neospora caninum* in Australia. *The Veterinary Journal* 188: 24-33.**

### **Intestinal and hepatic coccidiosis**

The coccidia identified in macropods are *Eimeria* spp., more than 40 of which have been described. Intestinal or hepatic coccidia associated with overt disease and lesions in macropods include *Eimeria kogoni* and *E. cunnamullensis*.

**Species affected:** Clinical disease with lesions attributed to intestinal and or hepatic coccidiosis is reported in eastern and western grey kangaroos; red kangaroos; and black-striped, tammar, whip-tail, red-necked and parma wallabies.

**Gross findings:** As in domestic animals, the gross changes seen in coccidiosis in wildlife may reflect the outcome of epithelial proliferation, inflammation with oedema or haemorrhage, or a combination of these changes. Lesions in kangaroos include subcutaneous oedema, copious serous fluid in body cavities, oedema and reddening of mesenteric lymph nodes, oedema of some viscera, and white or creamy-yellow foci (0.5 to 2 mm in diameter) visible through the serosa of the small intestine, especially the jejunum – these foci being more obvious in the mucosa which is eroded and haemorrhagic with blood stained exudates in the lumen in such areas. Oedema of the wall of the small intestine is present anteriorly but increases caudally, in association with luminal exudates changing from sero-mucoid to dark red, muco-haemorrhagic in the jejunum. Diphtheroid changes may occur in very severe cases. Lesions are less obvious in the ileum, and the wall of the large intestine is unaffected. Importantly, death may have occurred so suddenly that there was no history of blood stained faeces indicating severe haemorrhagic enteritis.

Lesions of coccidiosis involving both intestine and liver appear to be more common in tammar wallabies than in other macropods. In one study, hepatic lesions were white nodules 2 to 5 mm in diameter or pale areas surrounded by reddened borders. These lesions, which were scattered diffusely throughout the liver, were often associated with intestinal coccidiosis.

**Microscopic findings:** Especially in fatal coccidiosis in macropods, schizonts are quite conspicuous, and associated with pathological change. In one outbreak in wild eastern grey kangaroos only the small intestine was affected, and both small and large schizonts, and both micro- and macrogametes were present in cells of the lamina propria, sometimes with gametocytes 'of a different type' being present in epithelial cells. Large schizonts, that lay deep in the lamina propria were up to 350µm in diameter but were again arranged in clusters of 2 to 10 individual schizonts, the conglomerate having an overall diameter of up to 700 µm, and sometimes a rosette-like appearance. Microgametocytes in the lamina propria were scarce, but macrogametes were numerous. Rupture of large schizonts sometimes resulted in heavy infiltration of polymorphonuclear neutrophils. Both intestinal and hepatic lesions are expected in tammar wallabies with coccidiosis. Schizogony is seen initially in the small intestine but later it occurs in the liver.

### **Cryptosporidiosis**

The taxonomy of *Cryptosporidium* is dependent on molecular and biological characterisation, and is still in a state of flux. *Cryptosporidium* has been recognised, based on oocysts in faeces or stages in intestinal tissue sections, in a number of Australian marsupials, including the red, eastern and western grey kangaroos, the red-necked wallaby, the swamp wallaby, the yellow-footed rock wallaby, Tasmanian and red-necked pademelons, and the bilby. *Cryptosporidium fayeri* has been characterised from the koala, and *C. macropodum* from the eastern grey kangaroo; both species have been found in macropods. Other genotypes from Australian marsupials await further characterisation before they can be described as species. *C. muris*, probably originating in rodents, was detected in bilbies in a captive colony.

Gross abnormalities associated with *Cryptosporidium* infection include distension of the intestines with gas and watery fluid, and perhaps congestion. Microscopically there may be mild to moderate villous atrophy, crypt hyperplasia, some focal necrosis with loss of epithelium, and light to moderate infiltration of the lamina propria with predominantly mononuclear cells. Some metaplasia to low columnar, cuboidal or even squamous epithelium may occur.

Good cytology, necessitating minimal autolysis and therefore prompt fixation of infected intestine at necropsy, is required for ready recognition of cryptosporidia in histological sections. In sections stained with haematoxylin and eosin the parasites appear as basophilic bodies apparently attached to the surface of cells, sometimes giving the microvillus brush border a 'spotted granular appearance'.

They are spherical to elliptical in shape measuring 2 to 6 µm in diameter and protrude from the cell surface. Use of special staining, especially Ziehl Neelsen, may help recognition of oocysts within the gut lumen or faeces.

**Fayer, R. 2010. Taxonomy and species delimitation of *Cryptosporidium*. *Experimental Parasitology* 124: 90-97.**

**Power, M.L. Biology of *Cryptosporidium* from marsupial hosts. *Experimental Parasitology* 124: 40-44.**

### **Klossielliasis**

Cyst-forming protists of the genus *Klossiella* parasitise the kidneys of several macropodid species, as well as other marsupials. The presence of *Klossiella* is an incidental finding. There is no clear association of the parasite with clinical disease, and gross lesions are not apparent. Microscopically, interest centres on the parasite as almost always, an inflammatory reaction is absent or minimal. All stages of the life cycle of the parasite may be seen in sections of kidney and the nephron may be involved at various levels, necessitating a search especially of cortex but also contiguous medulla.

### **Leishmaniasis**

Infection with *Leishmania* sp. has been diagnosed as the cause of granulomatous dermatitis in captive red kangaroos, and subsequently in wild northern wallaroos, a black wallaroo and agile wallabies. Clinically, lesions presented as thickened, hairless and centrally ulcerated areas on the tail or ear, the margin of which was in one case deformed by the lesion. Lesions often began as hairless papules that became centrally encrusted, then later ulcerated as the crust fell away. Microscopic examination revealed hyperkeratosis, acanthosis, focal dermal necrosis and extensive granulomatous inflammation. The cytoplasm of macrophages within the dermis was seen to contain multiple round organisms with small basophilic, eccentric nuclei. Molecular studies have confirmed this organism as a probably novel species of *Leishmania*, which is transmitted by a day-feeding midge. The possible zoonotic significance of this agent is yet to be determined.

**Dougall, A., C. Shilton, J. Low Choy et al. 2009. New reports of Australian cutaneous leishmaniasis in Northern Australian macropods. *Epidemiology and Infection* 137: 1516-1520.**

**Dougall, A.M., B. Alexander, D.C. Holt et al. 2011. Evidence incriminating midges (Diptera: Ceratopogonidae) as potential vectors of *Leishmania* in Australia. *International Journal for Parasitology* 41: 571-579.**

### **Trypanosomiasis**

Most reports of *Trypanosoma* spp. infection in Australian native species relate to recognition of the parasite in blood smears, or more recently from PCR analysis of blood samples collected as part of broader surveys. Infected indigenous species include the eastern grey kangaroo, and (following experimental infection), the agile wallaby. In most of the reports, presence of trypanosomes was not associated with clinical disease or lesions.

Experimental infection of agile wallabies and dusky pademelons (*Thylogale brunii*) with *Trypanosoma evansi*, the agent of surra, endemic in south Asia, resulted in death or sickness, including anaemia. Gross lesions varied considerably but included pericarditis, serous atrophy of fat, splenomegaly, ulcerative gastritis and enteritis. Microscopically, there was mononuclear cell infiltration of the connective tissue of most organs with little cellular destruction – striking lesions being apparent in the choroids, heart, stomach and small intestine. In histological sections the trypanosome nuclei were seen as coccoid structures approximately 0.5 µm in diameter located in the interstitial tissue of many organs. Larger basophilic structures, 1 to 2 µm in diameter, and presumably representing more complete forms of the parasite, were occasionally seen within vacuoles in the stroma.

### **Other haemoprotist infections**

#### **Babesiosis**

There are reports of presumed *Babesia* infection in a rock-wallaby, unassociated with illness, and infection of an eastern grey kangaroo diagnosed by examination of a blood smear, then PCR. In the kangaroo – which recovered with treatment – there was a significant parasitaemia, and signs included dehydration, depression and anaemia (PCV of 21%).

**Haemoprotists in young eastern grey kangaroos.** Since 1994 a syndrome of severe anaemia associated with infection by an unidentified haematozoan has been recognised on the north coast of New South Wales. In addition to anaemia there is a tendency to bleed when attached ticks are removed. Pleomorphic intraerythrocytic parasites are seen in blood smears, and examination of histological sections reveals sequestration in a range of organs and variable formation of schizont-like forms within blood vessels, these latter changes being most marked in renal glomeruli, where schizont-like forms distend the capillaries of glomerular tufts.

#### **Other non-haematological protists**

These infections include steatitis associated with a coccidia-like protist in a red kangaroo, and giardiasis in several macropodid species.

## **5. HELMINTH DISEASES**

Information on helminth parasites of macropods is found in reviews and papers cited in Ladds (2009) as well as in the reviews and check-lists below. Beware: while the players don't change, their names sometimes do!

**Beveridge I. (1993). Marsupial parasitic diseases. In: M.E. Fowler (ed) *Zoo and Wild Animal Medicine, vol 3*. Saunders, Philadelphia. Pp. 288-293.**

**Beveridge, I., D.M. Spratt & P.M. Johnson. 2010. Diversity and distribution of helminths in macropodid marsupials. In: G. Coulsen & M. Eldridge (eds). *Macropods. The Biology of Kangaroos, Wallabies and Rat-kangaroos*. CSIRO Publishing, Collingwood. Pp. 231-242.**

**Spratt, D.M., I. Beveridge & E.L. Walter. 1991. A catalogue of Australasian monotremes and marsupials and their recorded helminth parasites. South Australian Museum, Adelaide. Pp. 105.**

### **(a) CESTODES**

#### ***Echinococcus granulosus***

Although infection with this cestode may cause debility or illness in an intermediate host, the disease complex, hydatidosis, is of particular interest because of its impact on spill-over domestic animal hosts and on account of its zoonotic implications.

The definitive host is the dingo or dog. Many macropod species are intermediate hosts for *E. granulosus*. Swamp wallabies may be especially susceptible, while in northern Australia the black-striped wallaby is a major intermediate host.

In macropodid intermediate hosts, hydatid cysts were found mostly in the lung and thoracic cavity, including attachment to the diaphragm, and less often in the liver and peritoneal cavity. Cysts were uni- or multilocular, and varied in size from less than 1 cm up to 10 cm in diameter. Multilocular cysts measuring 45mm have been observed to contain daughter cysts 10-18 mm in diameter. Disseminated infection with multiple small cysts in both thorax and abdomen has been reported. Unilocular cysts have been observed in the kidney, and in one rock wallaby with multiple hydatid cysts, it was estimated that less than 10% of pulmonary tissue was functional. Degenerate cysts were sometimes caseous or calcified.

Cysts in intermediate hosts can be categorised as fertile or sterile based on their microscopic appearance. In macropods the majority were fertile, with an inner, thin, germinal layer of small cells from which small hooked protoscolices might be seen budding, outside which was a distinct laminated cuticular zone. This in turn was surrounded by a tissue reaction consisting of a rim of histiocytes and perhaps some multinucleate giant cells, and an outer layer of fibrous tissue infiltrated by eosinophils, polymorphonuclear neutrophils, and lymphocytes. In involuting cysts, reduction of the cyst lumen was apparent, accompanied by thickening and folding of the cuticular laminae and prominent local fibrosis. Even in lesions in which involution is well advanced, at least fragments of the folded wall of the hydatid cyst remain and can be identified by their typical laminated, refractile appearance.

Barnes, T.S., A.W. Goldizen, J.M. Morton, et al. 2011. Cystic echinococcosis in a wild population of the brush-tailed rock-wallaby (*Petrogale penicillata*), a threatened macropodid. *Parasitology* 135: 715-723.

Barnes, T.S., L.A. Hinds, D.J. Jenkins, et al. 2011. Comparative pathology of pulmonary hydatid cysts in macropods and sheep. *Journal of Comparative Pathology* 144: 113-122.

#### ***Progamotaenia* spp.**

*Progamotaenia* spp. are found in the small intestine, where they are not associated with lesions, and in the bile ducts, where they are. Some species of *Progamotaenia* may be up to 1 metre long. Infection is reported in many macropodid species. Although species such as *P. effigia* and *P. diaphana* occur, *P. festiva*, is most often associated with lesions.

**Grossly**, up to 20 tapeworms may be found in the gall bladder and bile ducts, where they may cause cystic dilation of the major bile ducts, associated with masses of strobila with large proglottids. The bile duct walls in these areas typically are somewhat thickened.

**Microscopic examination** reveals a minimal inflammatory reaction, and changes associated with irritation of the mucosa by the parasites. There may be hypertrophy and hyperplasia of the mucosa of bile ducts and the gall bladder with resultant thickening, mild cholecystitis and cholangitis with a predominantly mononuclear cell infiltration, and usually slight fibrosis of bile ducts and surrounding tissue.

#### **Metacestodes**

Focal lesions in macropodid intermediate hosts caused by metacestodes of several tapeworms including *Anoploetaenia dasyuri*, *Dasyurotaenia* sp., and *Taenia (Multiceps) serialis* are described. These lesions present as pale 1-2 mm diameter foci in viscera or as subcutaneous cysts, with a thin fibrous wall, mild chronic inflammation, and within which a larval cestode is recognisable histologically.

### **(b) NEMATODES**

#### **Major alimentary tract nematodes**

The nematode parasites of Australian native mammals, particularly macropods, often are impressive in their prevalence, mass, magnitude and diversity. In the case of kangaroos, for example, up to 40 nematode species, all belonging to a single order, occur in the complex saccular fore-stomach of individual host species. In most cases, however, even heavy worm infestations are not detrimental to the host. Aspects of major gastro-intestinal nematode infections in macropods are as follows:

##### *Labiostrongylus* spp.

- Adults up to 12cm but only 3<sup>rd</sup> stage larvae pathogenic
- Larvae penetrate lymphoid tissue at junction of squamous and glandular parts of stomach to cause chronic sclerosing inflammation
- No overt clinical signs
- Gross – mucosal hypertrophy/hyperplasia
- Microscopic – if acute see many intralesional larvae associated with erosion; if chronic see 'chronic irritative hyperplastic' mucosal lesion

##### *Globocephaloides* spp.

- Not normally pathogenic
- Small (~ 1 cm dia.) worms suck blood
- In fatal cases (related to worm burden in juvenile animals) see worms in proximal small intestine associated with haemorrhage
- Microscopic – haemosiderin-filled macrophages in duodenal lamina propria

##### *Strongyloides* spp.

- Quite small worms (up to ~4 mm) present in stomach
- Only fatal in captive host animals
- Gross – erythema, oedema and haemorrhage, especially near pylorus. Mucosal hyperplasia apparent as folds or nodules
- Microscopic – severe erosion sometimes progressing to ulcers or abscesses. Larvae present in 'tunnels' in epithelium with caudal ends protruding into gut lumen

#### *Rugopharynx* spp.

- Adults up to 10 mm free in stomach lumen; probably only larvae pathogenic
- Gross – pale, firm nodules extend to junction of squamous and glandular epithelium. Larval heads in nodules with tail ends protruding into gut lumen
- Microscopic – fibroblastic reparative response. PAS-positive material around worm heads with associated necrosis & fibrosis. Larvae absent from more chronic lesions

#### **Other nematodes causing, or associated with lesions of the alimentary tract**

In addition to the major nematodes considered above, some other nematodes have been causally associated with lesions of the alimentary tract of macropods; these include *Cyclostrongylus* sp. (oesophagus), *Spirostrongylus* sp. (oesophagus and stomach), *Parazonialaimus collaris* (stomach), *Filarinema* spp. (stomach and intestine), *Hypodontus* sp. (small and large intestine), and *Paramacropostrongylus toraliformis* (colon and caecum). In most cases these nematodes are non-pathogenic.

#### **Hepatic and biliary nematodes**

Hepatitis caused by *Capillaria* sp., probably *C. hepatica*, is described in captive macropods. Macroscopically, irregular white nodules up to 5mm in diameter are scattered throughout the liver, and there may be adhesions between the liver and other viscera. Microscopically, aggregates of capillarial eggs, and sometimes adults are present in hepatic parenchyma. Early egg accumulation causes little cellular response, but later, connective tissue and mononuclear inflammatory cells progressively surround an infiltrate affected areas. Mineralisation, giant cell accumulation and extensive fibrosis may ensue, and in some late cases eggs may not be seen.

#### **Cardiovascular nematodes**

##### *Durikainema* sp.

- Small (< 3 mm) worms in blood and lymph vessels, especially in liver, epicardium
- In macropods regarded as an incidental finding (eastern & western grey kangaroo, eastern wallaroo, red-necked wallaby, agile wallaby, spectacled hare-wallaby, Tasmanian pademelon, Lumholtz's tree kangaroo)
- Gross – nodular yellow foci 1-2 mm diameter, especially on hepatic serosa
- Microscopic – depends on stage; at first have fragmentation of muscle layer of veins. Later obliteration of vein wall, recanalisation and increasing fibrosis; many eosinophils present

##### *Angiostrongylus cantonensis*

- Now reported in several macropod species
- History of nervous signs in red-necked wallabies (RNW)
- Gross – in RNW and rufous bettong – congestion and haemorrhage in brain or spinal cord associated with nematodes
- Microscopic changes in RNW and the bettong included haemorrhage and thrombosis, malacia, and severe eosinophilic meningoencephalitis associated with worms

##### *Breinlia* spp.

Large filarioid nematodes identified as *Breinlia ventricola* were described in free-ranging red kangaroos in Western Australia. Many worms were detected in the right ventricle and pulmonary arteries of apparently healthy animals killed in a game meat export abattoir. Length of worms was up to 22 cm and 26 cm for male and female worms, respectively.

*Breinlia woerlei* are worms 14 to 18 cm in length that occur in the right ventricle and cysts in the lung of narbarlek and the short-eared rock-wallaby. Large pulmonary cysts may compromise respiration.

#### **Body cavity nematodes**

##### *Breinlia mundayi*

As well as being intravascular nematodes, several species of *Breinlia* occur within the body cavities of various macropodid species, possibly causing mild serositis with consequent fibrin tags or adhesions – sometimes being sufficiently extensive to cause constrictive pericarditis. In *B. mundayi* infection of swamp wallabies, microscopic examination revealed mild eosinophil infiltration and fibrosis on the surface of many organs, including lungs, heart, spleen, liver and kidneys. Calcified nematodes were

also found encapsulated in the omentum. Eosinophil infiltrations around blood vessels in the heart, hepatic portal tracts and renal cortex were considered to be reactions to circulating microfilariae. Focal granulomas in red pulp along the margin of the spleen were associated with sequestered microfilariae.

#### *Ophidascaris (Amplichaecum) robertsi*

This ascarid occurs in pythons, and uses small mammals as intermediate or paratenic hosts, where infective larvae develop. Migration of *Ophidascaris* larvae, especially in the liver, was considered the cause of death in recently captured musky rat-kangaroos.

### **Connective tissue nematodes**

#### *Pelecitus roemeri* (formerly *Dirofilaria roemeri*)

- Found in at least 5 genera of macropods
- Appear not to cause illness
- Large nematodes – up to 14 cm in length
- Gross – in subcutis, especially in vicinity of knee. May be free in fascia or encapsulated. Depending on stage of infection, discrete hard 'pellets' may be seen
- Microscopic – changes vary between species but include granulomatous inflammation, mineralisation, and viable and degenerating microfilariae

#### *Breinlia annulipapillatum*

This filarioid nematode has been identified in the subcutis of black-striped wallabies, with splenic granulomas surrounding degenerating microfilariae.

### **Respiratory nematodes**

The significance of lung parasites to the health of marsupial hosts is poorly understood. The list of parasites is long, but most infections do not cause overt disease, and in the majority of cases, associated lesions are minimal.

#### ***Marsupostrongylus* spp.**

A number of *Marsupostrongylus* spp. and related metastrongyles occur in the respiratory tracts of many, diverse marsupials including many macropods. These lungworms are found mostly in the terminal bronchioles but are reported in the alveoli, larger bronchioles, bronchi and trachea. Clinical signs attributable to *Marsupostrongylus* seem to be rare and most lesions due to infection are microscopic.

#### ***Capillaria* spp.**

In respiratory capillariasis the nasal sinuses or lungs may be affected. Infection has been reported in bettongs, and potoroos. Gross lesions in uncomplicated cases were confined to the lungs, which contained many pale yellow-grey slightly raised foci 1 to 5 mm in diameter containing thick brown mucus, and scattered throughout the parenchyma of all lobes.

Microscopically, lesions mainly were centred on airways. There was bronchiectasis, and accumulation of necrotic debris, desquamated epithelial cells, polymorphonuclear neutrophils, and nematode eggs within bronchial and bronchiolar lumina. Nematodes were located on or within the bronchial and bronchiolar epithelium, causing erosion and necrosis. Infiltrates in bronchiolar epithelium and to a greater extent in the lamina propria, muscle layer and adventitia, were dominated by lymphocytes, macrophages and plasma cells, but not eosinophils.

### **(c) TREMATODES**

In contrast to cestodes and nematodes, trematode infections of most macropods are uncommon, no doubt related to habitat and presence of the required intermediate hosts. Except fascioliasis, reports of their causing disease are rare.

#### **Fascioliasis**

Macropods infected with *Fasciola hepatica* include kangaroos, wallabies, and pademelons.

- Infected macropods may have no clinical signs, or may show severe cachexia and anaemia. Even when large numbers of flukes are present, however, signs of illness may not be apparent
- At necropsy, up to 80 flukes have been counted in red-necked wallabies
- Changes associated with flukes have included severe distortion of the liver with excessive fibrosis, and cystic swelling of the bile ducts
- Microscopic changes in fascioliasis clearly vary with host susceptibility or resistance. Mild to severe cholangiohepatitis is the essential lesion. Widespread multi-focal haemorrhage and necrosis of hepatic parenchyma due to migrating immature flukes may occur – with subsequent scarring, and eosinophil infiltration around bile ducts.

### **Paramphistomiasis in agile wallabies**

Two paramphistomes, *Macropotrema pertinax*, and *Gemmellicotyle wallabicola* may cause gastrointestinal lesions in the agile wallaby but these are of a localised nature and seem to be of little consequence to health of the host.

## **6. ARTHROPOD DISEASES**

### **(a) Pentastomes**

Mammals, including humans, may serve as both definitive and intermediate hosts for pentastomes, worm-like arthropod parasites. A single pentastome nymph was detected at post-mortem of a free-ranging bridled nailtail wallaby.

### **(b) Mites**

#### ***Sarcoptes***

Sarcoptic mange has been reported in agile wallabies and a wild swamp wallaby, among macropods. *Sarcoptes* infection is a zoonosis.

- Lesions in wallabies had varied from generalised over much of the body, through involving mostly anterior parts of the body, to affecting mainly the hindlimbs, tail and caudal trunk. Lesions consisted of thick crusts, sometimes with deep fissures.
- Histopathology of sarcoptic mange lesions was characterised by a thickened epidermis, marked hyperkeratosis with frequent mites deep within the keratin layer of the epidermis, and a predominantly mononuclear cell accumulation in the dermis. Mite eggs and bacterial colonies were seen superficially associated with an infiltrate of mixed inflammatory cells.

**Holz, P.H., G.M.B. Orbell & I. Beveridge. 2011. Sarcoptic mange in a wild swamp wallaby. Australian Veterinary Journal 89: 458-459.**

#### ***Diaboliocoptes***

*Diaboliocoptes*, formerly known only from the faeces of a Tasmanian Devil, is a sarcoptiform mite which, in concert with lice (*Heterodoxus wolabati*), was associated with dermatitis in free-ranging swamp wallabies. Signs included alopecia and hyperkeratosis of the pinnae and tail, over lateral parts of the body and at the tail base, inguinal area and inner aspects of the thighs. Acanthosis, hyperkeratosis and superficial predominantly lymphoplasmacytic perivascular dermatitis were evident, complicated by pyoderma and superficial dermatophytosis in some areas. Mites (*Diaboliocoptes*), eggs and pigmented debris were in hair follicles on the inner aspects of the thighs.

**Portas, T.J., A. Crowley & J. Hufschmidt. 2009. Ectoparasitic dermatitis in free-ranging swamp wallabies (*Wallabia bicolor*) in New South Wales. Australian Veterinary Journal 87: 160-162.**

#### **Trombiculid mites**

- An extensive moist pustular dermatitis affecting the inguinal, abdominal and axillary regions of a wild-caught yellow-footed rock-wallaby was associated with mites identified as *Odontacarus (Leogonius) adelaideae*. Microscopic examination of tissue from the edge of a lesion revealed mites within invaginations of the epidermis – the infolded, attenuated epidermis being lined by a laminated eosinophilic to hyaline membrane (stylostome), with a mite located in the lumen. The dermis surrounding the stylostome was heavily infiltrated by

polymorphonuclear neutrophils, many eosinophils and mononuclear cells, especially macrophages.

- Heavy infections of grey kangaroos with a related trombiculid mite, *Trombicula sarcina* (also called *Eutrombicula sarcina*) caused shallow crater-like ulcers on the inner surface of the thighs. The colonies of mites appeared as orange coloured patches – sometimes arranged as a short chain of lesions each about 6 mm in diameter and harbouring hundreds of mites. Such lesions, however, did not appear to cause irritation – such as occurs in human trombidiosis.
- Trombiculid mites identified as *Eutrombicula hirsti* were commonly found embedded in, and associated with dermatitis of skin – again on the medial aspects of the thighs – of wild agile wallabies. Surrounding the attachment site of mites the skin was inflamed for up to 5 mm, with a central depression containing encrustations.
- In a study of parasites on free-ranging, endangered bridled nailtail wallabies, four species of trombiculid mites (*E. hirstii*, *Odontacarus* sp., *Guntheria philippensis*, and another *Guntheria* sp.), located in the inner aspect of the pinna and inner thighs were associated with scab formation, local erythema and alopecia. *E. hirsti* were closely associated with the skin surface, which was hyperkeratotic, with lymphoplasmacytic and eosinophilic infiltrates in the dermis.

**Old, J.M., J. Lowrey & L.J. Young. 2009. Scub-itch mite infestation in the endangered bridled nailtail wallaby. Australian Veterinary Journal 87: 338-341.**

#### **Dermanyssid mites**

The dermanyssid mite, *Thadeua serrata*, has been identified as the likely cause of dermatitis in free-ranging Proserpine rock-wallabies and black-striped wallabies. Clinically, lesions were present on the sparsely haired axillary and inguinal regions of all wallabies examined, with lesions being more conspicuous in the inguinal area. These lesions were crateriform, and approximately 5 mm in diameter with a central pit. In heavy infestations, lesions were confluent and involved much of the hairless areas. In some instances the application of external pressure resulted in the extrusion of mites.

Microscopically, the crateriform lesions containing mites were seen to extend into the superficial dermis. The crater walls were hyperkeratotic, and within the dermis there was a mixed inflammatory infiltrate composed predominantly of polymorphonuclear neutrophils, eosinophils and lymphocytes.

#### **Other cutaneous mites**

##### ***Cytostethum* sp.**

Mild superficial lesions in trapped long-footed potoroos, with hair loss and abrasions due to pruritis, were associated with *Cytostethum* sp. mite infection. Health of infected animals did not appear to be adversely affected and lesions resolved after several months.

#### **(c) Ticks**

It is unclear to what extent hypersensitivity-allergic reactions to ticks occur in native animals; the severe local and systemic responses that have been seen in man are not reported but there is one early record of neurological signs being observed in a kangaroo immediately after an engorged kangaroo tick (*Ornithodoros* [syn *Argas*] *gurneyi*) had dropped from it.

In other studies, pet kangaroos and wallabies were considered to have succumbed to tick paralysis. The occasional cases of tick paralysis in wildlife species that are normally regarded as immune, have given rise to considerable speculation on the nature of this immunity. At least three species of tick, namely *Ixodes holocyclus*, *Ixodes cornuatus*, and *Ixodes hirsti* have been incriminated as definitely causing tick paralysis in Australian native mammals.

Lesions have not been described in native species with tick paralysis, but presumably findings in most cases were those attributable to ascending paralysis with secondary infection, especially bacterial pneumonia.

In red kangaroos shot in the wild, blemishes – so-called ‘pocks’– resulting in the commercial downgrading of skins, were attributed to infestation with *Amblyomma trigutatum*. Grossly, the lesions were considered to be attachment sites.

#### **(d) Fleas**

A flea identified as a primary or contributing cause of lesions in a range of captive macropods is the stickfast flea *Echidnophaga* spp. Animals with large flea burdens are usually stressed, debilitated, or anaemic, perhaps with other concurrent disease.

Stickfast fleas in macropods were observed to be located on the inside of the ears, around the muzzle, on the supraorbital ridges or tarsus, at the base of the neck, or on the back between the shoulder blades. The presence of fleas was directly associated with marked alopecia, and in some cases there was also slight excoriation of the skin. It was presumed these lesions were due to irritation with scratching and self-mutilation.

#### **(e) Other arthropod ectoparasites**

- In bridled nailtail wallabies, the presence, over much of the body, of large numbers of the louse, *Heterodoxus* sp., was associated clinically with frequent scratching, matted fur, bare skin patches and wounds.
- Larvae of the kangaroo bot-fly *Tracheomyia macropi* have been recorded in the trachea and occasionally the bronchi and bronchioles of kangaroos and the wallaroo. Compromised respiratory reserve with severe infestation may render animals more prone to predation. Grossly, the larvae move freely over the mucosal surface, but if an attempt is made to dislodge a living specimen, the ‘firmness of the attachment is surprising’. Burdens of 2 to 10 larvae per animal are recorded and there may be associated erythema of mucosa and slight ulceration. Microscopic changes include mucosal ulceration, mononuclear cell infiltration of the submucosa, and squamous metaplasia and fibrosis of tracheal mucosa.
- Overseas, kangaroos and a wallaby in a zoological garden suffered *Cuterebra* sp. myiasis over a two month period during late summer – early autumn. Discharging lesions were located over the thorax or on the lateral aspect of a hindlimb. *Cuterebra* spp. are not reported in Australia.
- Severe infestations of kangaroos, wallabies and the wallaroo, with the sandfly *Austrosimulium pestilens*, have been the cause of intense irritation. Large numbers of sandflies in the facial area were associated with almost complete closure of the eyelids and marked swelling of surrounding areas. There was also oedema of the jaws and muzzle, which in some animals were denuded of hair and were inflamed, presumably as a result of scratching.
- Other reports of fly worry in kangaroos and wallabies include bites by a number of hippoboscids flies.

## **7. DISEASES CAUSED BY EXOGENOUS TOXINS**

### **Lead poisoning**

All species are susceptible. Lead poisoning was diagnosed as the cause of death of an eastern grey joey that had lead shot embedded in its musculature, although in most species lead is not absorbed from embedded shot. The joey developed a severe microcytic non-regenerative anaemia and lead concentration in the liver was 13 ppm. Lesions were not reported.

Gross changes, other than injuries secondary to nervous dysfunction and resulting trauma, are not observed in animals with lead poisoning.

### **Fluorosis**

Fluorosis caused illness and deaths of kangaroos inhabiting heath and farmland surrounding an aluminium smelter.

Grossly, there was severe osteophytosis on the distal tibia and distal fibula, on the periarticular aspects of the calcaneus, and the distal calcaneal process. In one kangaroo, which also had extensive osteophytosis and ankylosis of coccygeal vertebrae, the fourth metatarsus was fused to the

tarsal bones. In joints, the surface cartilage was yellow and was moderately fibrillated, with focal areas of cartilage loss. Marked longitudinal grooves were apparent on the joint surface, and several chondromas had developed at the transitional zone of the synoviae. The upper incisors of affected kangaroos were variably roughened, dull and stained rather than being smooth, shiny and white. Focal enamel hypoplasia, and in one animal abnormal wear, was apparent. The upper and lower molars were also worn unevenly.

Histopathological examination of a range of tissues from affected kangaroos essentially revealed only incidental changes. Bone fluoride levels for animals of comparable age were elevated in the population near the smelter, in comparison with a control population.

**Clarke, E., I. Beveridge, R. Slocombe et al. 2006. Fluorosis as a probable cause of chronic lameness in free-ranging eastern grey kangaroos (*Macropus giganteus*). *Journal of Zoo and Wildlife Medicine* 37: 477-486.**

**Husfchmidt, J., I. Beveridge, G. Coulson et al. 2011. Bone fluoride concentrations of eastern grey kangaroos (*Macropus giganteus*) resident near an aluminum smelter in south-eastern Australia. *Ecotoxicology* 20: 1378-1387.**

### **Pesticide toxicity**

Organochlorine poisoning is reported in kangaroo joeys that were immersed (except for the head and neck) in a 1% DDT emulsion for a few seconds to control a severe infestation with the stickfast flea (*Echidnophaga gallinacea*). The first signs of toxicity were observed some hours after immersion and included intermittent convulsions, high jumping, 'thrashing around', terminal muscle twitching and death within an hour of showing initial nervous signs. Necropsy findings were not reported.

### **Fluoroacetate poisoning**

Deaths of wildlife species may occur either as a result of ingestion of sodium fluoroacetate (compound 1080) used to control vertebrate pests, or after eating fluoroacetate-containing plants such as heartleaf (*Gastrolobium* spp.). In Western Australia, several species of mammal, including western grey kangaroos, have been observed to be tolerant to fluoroacetate, indicating an evolutionary adaption to this toxin in plants.

Clinical signs of 1080 poisoning are typically seen about 30 minutes to three hours following ingestion, and death may occur within hours or after several days. In most studies of 1080 toxicity, lesions – due presumably to heart failure – were either not present or not described.

### **Anticoagulant poisons**

Second generation anticoagulant vertebrate pesticides such as brodifacoum may kill an animal after one feeding. They are considerably more toxic than first generation anticoagulant pesticides such as warfarin (Ratsak<sup>®</sup>) or pindone, which require multiple feedings in order to kill.

Reports of pindone poisoning in free-ranging native mammals in Australia include western grey kangaroos. Based on observations in dogs poisoned with first generation anticoagulants, signs do not occur until several days after exposure and include weakness, pale mucous membranes, epistaxis, vomiting of blood and rectal bleeding. Blood may be coughed up and haemorrhages may be apparent in the skin or on mucous membranes. Lesions seen at necropsy include the pallor of anaemia, with haemorrhage – ranging from petechiae to major extravasations – in or on various organs, and perhaps into the gut.

### **Plant poisonings**

- Yew (*Taxus baccata*) poisoning is described in red-necked wallabies which died within 24 hours of ingesting leaves of the plant. Necropsy revealed congestion of kidneys, myocardium, liver and lungs, and acute enteritis was apparent. Microscopic lesions in the brain were indicative of circulatory disturbance.
- Rhododendron (*Rhododendron* spp.) toxicity was suspected in a young, hand reared, western grey kangaroo which was observed eating the plant several hours before initial signs developed. Signs were suggestive of gastrointestinal pain and spasm. After treatment with a

range of agents over the following week the animal returned to normal. Lesions were not reported.

- *Lantana camara* poisoning in red kangaroos captive overseas resulted in hepatotoxicity with secondary photosensitisation. At necropsy, in addition to jaundice, the liver was enlarged with pale yellow to red-yellow mottling. Microscopic changes included hepatocellular enlargement with vesiculation of the nuclei, and sporadic feathery degeneration of the cytoplasm.
- Also in red kangaroos, free-ranging in areas where pyrrolizidine-containing plants occur, hepatitis typical of pyrrolizidine alkaloidosis has been observed.
- *Parsonsia straminea* (monkey rope, silk pod), a woody climbing vine, was associated with ataxia, grinding of teeth and cerebral oedema in eastern grey kangaroos kept in an enclosure in south-east Queensland.
- Neuronal pigmentation characteristic of chronic phalaris poisoning is recorded in captive red kangaroos and wallabies grazing phalaris-dominant swards.
- Poisoning following ingestion of the popular hedge and garden plant *Duranta erecta* (golden dewdrop, Sheena's Gold, Geisha Girl) is reported in kangaroos. Signs were those of drowsiness. Lesions were not described; in (sometimes fatal) poisoning in the dog and cat there may be gastro-intestinal haemorrhage with melena.

### **Oxalate poisoning**

Oxalate nephrosis of suspected plant origin or of unknown cause or pathogenesis, is reported in a swamp wallaby and in Gilbert's potoroos (see under diseases of unknown aetiology) respectively.

Gross examination of the wallaby revealed stippling of the surface of the kidney, with 'streakiness' of the cortex apparent on the cut surface. Microscopically, masses of oxalate crystals were in distended tubules but the associated inflammatory response was minimal and there was no evidence of fibroplasia. It was concluded that the likely source of oxalate was fresh-cut kikuyu grass (*Pennisetum clandestinum*), that possibly was also contaminated with soursob (*Oxalis pes-caprae*).

### **Mebendazole toxicity**

Following ongoing deaths of macropods due to a hitherto undescribed haemorrhagic septicaemic syndrome (HSS) characterised by an absence of a neutrophilic response to bacterial infection, the possible role of prior dosing of animals with mebendazole was investigated in pademelons.

Lesions observed at necropsy included haemorrhages in many organs including lungs, heart, liver, muscle and gastrointestinal tract – which was ulcerated in one location and associated with peritonitis.

Microscopically, there was diffuse ulcerative necrosis of intestinal mucosa with associated proliferation of bacterial rods. A mild, non-suppurative inflammatory response was apparent. Bone marrow from the tibia was haemorrhagic and necrotic, with severe depletion of haemopoietic cells. Bacteria were observed in bone marrow and lymphoid tissue in separate animals and a range of opportunistic enteric bacteria was cultured from blood or internal organs. It was concluded that illness and deaths were due largely to a compromised inflammatory response and that macropods were probably more sensitive to the effects of mebendazole on bone marrow than humans and other animals.

## **8. NEOPLASIA**

For neoplasms and proliferative lesions submitted from 402 terrestrial mammals to the Australian Registry of Wildlife Health between 1974 and late 2005, a total of 43 (~ 11%) were from macropodids. Some information on these neoplasms is presented in Table 1.

Table 1 - Final or provisional diagnoses of archived cases of neoplastic-proliferative lesions in macropodids in the Australian Registry of Wildlife Health

GENERA OR SPECIES	TYPE OF NEOPLASM		
	MESENCHYMAL	EPITHELIAL	HISTOGENESIS – OTHER OR UNSPECIFIED
Kangaroo (12)*	Lymphoma - lymphosarcoma; mastocytoma; osteosarcoma	Trichoepithelioma; adenocarcinoma, (of mammary gland, and bronchiogenic); squamous cell carcinoma (of pancreatic or bile duct); carcinoma, cholangiocellular	Melanoma (of pouch); amelanotic melanoma
Wallaby (18)	Lymphoma – leukaemia; possible hamartoma (of liver); haemangiosarcoma; carcinoma, renal	Probable hepatoma; adenocarcinoma (of mammary gland – metastatic to liver and lung); adenocarcinoma, cutaneous, and probable hepatic); squamous cell carcinoma; papilloma	Nephroblastoma
Bettong (4)	Lymphosarcoma; angioma (of spleen and of liver);	Carcinoma (of duodenum)	
Wallaroo (3)		Adenoma (of thyroid); adenocarcinoma (biliary, and probable mammary)	
Pademelon (3)	Lymphoma - leukaemia	Carcinoma, bronchiogenic	Melanoma (of eyelid)
Quokka (2)	Liposarcoma	Adenocarcinoma, of mammary gland	
Potoroo (1)	Fibrosarcoma, metastatic		

\* = number of cases

In addition, as well as all of the above neoplasms or proliferations in macropodids, a review of published cases – many from overseas zoos – revealed that only a minority were mesenchymal, the overwhelming majority being epithelial. These published cases, grouped according to histogenesis, are as follows:

**Mesenchymal** - Vasoformative proliferations – probably hamartomas, blast cell lymphoma, lipomatosis, osteochondromatous proliferation, and generalised sarcoma.

**Epithelial** - Papilloma of tongue, squamous cell carcinoma (SCC) of the oral cavity or gastric fundus, pulmonary adenocarcinoma, hepatoma, adenomatous proliferation of the intrahepatic duct, SCC of the cervix and vagina, adenocarcinoma of the oral cavity, squamous tumour of the stomach, thyroid adenoma, and an early report of primary and metastatic lung tumours.

**Other histogenesis** - Melanoma of the oral cavity.

Collectively, the above observations and reports emphasise the broad range of neoplasms that occur in captive macropodids. Apart from papillomas associated with poxvirus, however, neoplasms in free-ranging macropods are apparently uncommon. Most of the above reports of neoplasms give diagnosis but provide little detail on gross or microscopic findings – presumably because features typical of tumour type – and therefore used to support diagnosis, were those found in comparable tumours in domestic animals and man.

## 9. CONGENITAL, GENETIC AND POSSIBLE INHERITED DISEASES

There are few reports of congenital and perhaps inherited disease in macropodids, and little information on lesions present. Table 2 lists some reported conditions.

Table 2 - Some changes or diseases of congenital, genetic or suspected inherited origin, in Australian macropodids

Species	Change, lesion or disease	Comment
Eastern grey kangaroo & agile wallaby	Presumed ectopic parathyroids	Observed histologically within cervical thymus of 11 animals. No associated changes observed
Eastern grey kangaroo	Unilateral failure of development of premolars and molars	Aplasia of premolars & molars in right mandible; presumed congenital. Left mandible normal except for 'lumpy jaw' lesion – perhaps due to unilateral mastication; <i>Bacteroides</i> sp. was isolated
Tasmanian pademelon	Syndactyly in manus of pouch embryo	External examination revealed bilateral lesions, with digits 3 and 4 conjoined partially or completely on right and left sides, respectively
Red kangaroo	Multiple genital anomalies	Aplasia of penis, pouch and teats. Testes status not ascertained, but assumed absent. Animal had XX karyotype
Agile wallaby & eastern grey kangaroo	Intersex	Various abnormalities involving penis, pouch, teats and scrotum. Testes status not ascertained but assumed present in wallaby and absent in kangaroo. Karyotypes: wallaby XXY; kangaroo XX
Red kangaroo, wallaroo, tammar wallaby, quokka	Intersex	Variable presence, location and/or development of pouch, mammary tissue, scrotum and testes. Karyotypes of affected animals included XO, XXY and XXY/XY/XX, and XY

## 10. NUTRITIONAL AND METABOLIC DISEASES

### (a) Generalised nutritional excess or deficiency

In Western Australia, quokkas on Rottnest Island suffered severe starvation, weight loss and significant mortality during the summer months. Illness and mortalities were associated with increased excretion of *Salmonella* spp. considered to be related in turn to digestive physiology altered by poor quality feed during summer.

Pathological changes in adult kangaroos and other macropods dying during drought are inadequately studied but include lumpy jaw in red kangaroos, dehydration and cachexia, and a seasonal anaemia associated with reduced protein in vegetation. Other morphological changes in drought affected red kangaroos include decreased or increased size of the pituitary glands in adult males and females, respectively, and in both sexes a decreased size of adrenal glands accompanied by cortical folding and nodulation – with indications in females of continued stimulation of the zonae fasciculata and glomerulosa, perhaps reflecting better adaptation. Nutritional deficit due to drought, especially if combined with high environmental temperatures, has been shown by histology to cause testicular degeneration with diminished fertility of red kangaroos in central Australia.

As well as the influence of quantity and quality of food offered to captive wildlife, the form in which it is offered may also impact on health. Uroliths (composed of calcium oxalate and or phosphate) were found in a range of captive marsupials including eastern grey kangaroos, and a red necked wallaby.

### **(b) Deficiency or excess of specific dietary components**

**Vitamin D** - A deficiency of vitamin D may manifest as diminished provisional calcification of bone leading to pathological fractures of bone as in rickets, with persistence of cartilage. Osteodystrophies due to vitamin D<sub>3</sub> deficiency or calcium and phosphorus imbalance are not uncommon in orphaned pouch-young marsupials and are generally seen as deformities of the thoracic cage.

**Vitamin E** - (but not necessarily selenium) responsive nutritional myopathy has been observed in a range of macropods. Other disorders possibly linked to vitamin E include a deficiency-related steatitis in a yellow-footed rock wallaby, and the development, in kangaroos given large doses of vitamin E, of scaly yellow cutaneous plaques that eventually regress.

At necropsy, in animals with nutritional myopathy there is typically pallor and atrophy of pelvic and femoral muscles which may have a spotted yellow-brown discoloration, or may, in severe cases, be firm, gritty and friable. Other muscles such as those of the thorax and spine may also be affected.

Microscopically, changes in quokkas were especially apparent in pelvic muscles, and consisted of degeneration and necrosis. Swelling of fibres was apparent in early stages and sarcolemmal nuclei were pyknotic in some areas but plump and proliferating in adjacent viable zones. In older lesions macrophages were filled with necrotic debris, and small numbers of polymorphonuclear neutrophils were present.

**Sodium** - Sodium deficiency was described in eastern grey kangaroos free-ranging in the Snowy Mountains alpine region. Clinically, affected animals exhibited salt-hunger, and at necropsy the adrenal glands were observed to be about double the weight of adrenal glands of 'control animals from the sea coast'. Microscopically, the zona glomerulosa was expanded, and the salivary glands of sodium deficient kangaroos had a more extensive duct system. Also, cells lining the ducts were increased in height – these salivary gland changes indicating chronic hyperactivity in active sodium resorption.

**Lactose excess or intolerance** - A dietary excess of lactose in hand-raised orphaned pouch-young marsupials may be associated with diarrhoea or cataracts. Whether or not, and to what extent cataract formation in a range of marsupials is due to primary or acquired deficiencies of galactokinase and transferase has yet to be resolved. Although the cause is not understood, cataracts appear to be more common in joeys fed milk which contains lactose. Cataracts – many considered to be of nutritional origin – have been described in a range of captive orphaned macropods, as well as wild swamp wallabies. In marsupials, cataracts considered due to enzyme deficiency are bilateral. Clinically, opacities may be associated with a disordered gait and disorientation consistent with visual deficit. With mature cataracts the suspensory lens ligaments are weak so that prolapse of the lens readily occurs – rendering surgery 'almost impossible'. Glaucoma is a frequent sequel to prolapse. Also, in such cases the primary vitreous may also be opaque.

### **(c) Metabolic diseases**

Diabetes has been induced in the red kangaroo by chemical destruction of the islets of Langerhans. Affected animals showed negative nitrogen balance and hyperglycaemia, and became inco-ordinated and comatose. Insulin restored normal metabolism.

Renal failure associated with severe, fatal nephrocalcinosis in the endangered Gilbert's potoroo is described in Western Australia. It was suggested that the cause may be an inherited metabolic defect.

## **11. PHYSICAL EXERTION, TRAUMA, PREDATION AND MISCELLANEOUS INJURY**

### **Capture myopathy**

Although seen in many wild species including birds, capture-, or so-called exertional myopathy in Australia – sometimes due to trapping – is mostly diagnosed in macropods.

The cause of capture myopathy is debated and the disorder needs to be differentiated – largely on the basis of history – from nutritional muscular dystrophy. Signs are usually apparent one to two weeks post-capture with deaths occurring anytime up to four weeks post-capture.

Signs include stiffness, pain and spasm of muscle groups especially in the cervical region, torticollis, partial paralysis of the limbs, posterior paralysis and prostration. Respiration is laboured and heart rate increased. Myoglobinuria may be present. Sometimes animals die suddenly from acute heart failure without any premonitory signs.

Macroscopically, muscle lesions occur principally in the limbs. In macropods, the neck muscles are frequently involved, as are muscles of the back. Lesions are bilateral but not necessarily symmetrical. Affected areas are well delineated, softer than normal and are slightly pale to light grey-brown with occasional haemorrhage. Similar lesions may be found in myocardium. The earliest muscle lesions are detectable 1-2 hours post-capture. At 10 hours the affected muscles are dark red and dry and may appear as streaks or involve the whole muscle. At 3-4 days lesions become paler, soft and gelatinous. After one or more weeks there is increasing fibrosis and affected muscles become white and firm.

Microscopically, in the early stages there is loss of cross striation of muscle, hyaline eosinophilia and development of contraction bands and myofibre fragmentation, sometimes with mineralisation of mitochondria. Later an inflammatory infiltration of neutrophils and macrophages occurs and in more advanced lesions macrophages and proliferating sarcolemma predominate. Fibrosis and mineralisation of necrotic fibres may also be observed. Microscopic lesions in other tissues may include myoglobinuric nephrosis with the presence of casts, pulmonary oedema and congestion, periacinar hepatic necrosis due to anoxia, lympholysis, pulmonary congestion and oedema if the myocardium is affected, perivascular haemorrhages in the brain and meninges, and sometimes haemorrhage and necrosis in the adrenal cortex.

### **Inter-specific trauma and predation**

In addition to those injuries inflicted by man as the major predator, lesions attributed to native or introduced predators are described in many Australian native mammals. Necropsy examination of mostly juvenile red kangaroos attacked by dingoes revealed animals were in the majority of cases found in right lateral recumbency, usually with the head slightly thrown back. Unless the carcass had been eaten, signs of injury were unusual although occasionally fur at the throat was slightly blood-tinged. Examination of the skinned carcass, however, presented a 'very different picture', as extensive bite wounds were always present over the neck and thorax, or throat. These lesions were deep penetrating and accompanied by subcutaneous and intramuscular haemorrhage, and oedema. Penetration often extended to major organs of the thorax, sometimes the abdomen also, and was accompanied by fractures of bones such as ribs, scapula and vertebrae. Bite wounds were also common in muscles of the left hindquarter. Similar internal and external lesions were observed in captive juvenile macropods attacked by dogs.

### **Motor vehicle induced trauma**

There appear to be no published analyses of lesions induced in macropods by motor vehicle accidents. A five year study of road-kills of eastern grey kangaroos and swamp wallabies on a highway in Victoria, however, revealed that the majority of kills were of adult males, and interestingly, that most road-kills occurred around the time of full moon, suggesting that kangaroos are more mobile during that phase.

### **Drowning**

Drowning was confirmed or suspected as the ultimate cause of death of wild kangaroos that were blind as a consequence of orbivirus infection. Gross and or microscopic changes in these cases variously included marked pulmonary oedema with froth-filled airways, emphysema and atelectasis, and diatoms present in affected lung tissue. Presumably, as in other species the possible occurrence of so-called 'dry drowning' – in which no fluid is present in airways – needs to be kept in mind.

### **Gunshot and foreign body injury**

Gunshot wounds are recorded in macropods including a red-legged pademelon joey with multiple pellets in several sites, and an adult eastern grey kangaroo with streptococcal osteomyelitis for which the initial injury was considered, on the basis of radiography, to have been fracture of mid-shaft radius and ulna by penetration of tissue by a high velocity projectile. Such projectiles are normally copper jacketed with a small soft lead point. It was suggested that on striking the area the force of impact would have fractured the bones then passed through interosseous space leaving very small scattered flakes of lead as observed in this case. In contrast, in gunshot injuries caused by low velocity projectiles, larger lead particles are observed.

Foreign bodies, especially string, are common causes of intestinal problems in pet macropods.

### **Excessive cold or heat**

Hairless pouch-young marsupials may be regarded as being largely poikilothermic so that in macropod joeys both hyperthermia (body temperature  $> 38^{\circ}\text{C}$ ), and hypothermia (body temperature  $< 35^{\circ}\text{C}$ ), may occur with inappropriate housing or handling.

Gross changes observed in an agile wallaby joey that died of heat stroke included petechiae and ecchymoses in both cardiac ventricles, marked congestion of the lungs, and apparent cerebral oedema such that the sulci of the cerebral hemispheres were less obvious than normal and the gyri protruded through cranial incisions during dissection. Microscopic examination confirmed cardiac haemorrhages in endocardium, myocardium and epicardium, and haemorrhages were also seen in many other tissues including stomach and intestines, kidney, adrenal, thymus, and cerebellum. Vascular congestion was apparent in all organs. Other changes included multifocal myocardial necrosis, occasional necrotic foci in the liver, and brain lesions such as oedema, and loss of Purkinje cells.

Together with drought, high environmental temperatures were shown to cause testicular degeneration in wild red kangaroos in central Australia. With chronic degeneration in some aged males – presumably the combined result of repeated hot weather and nutritional deficit – the testes were brownish, and smaller than normal. Microscopically, spermatogenesis was in abeyance in some seminiferous tubules in these animals; testicular stroma was comparable to that in younger (but nevertheless mature) animals.

In the testes of kangaroos sampled in summer, impaired spermatogenesis was manifested by reduced diameter of tubules with vacuolation and lack of germinal epithelium, the presence of large multinucleate cells, and cellular debris within the lumens of tubules and the efferent ducts. Quantitative histological studies of the testes of these animals also revealed differences in the interstitium, especially in relation to drought. In times of dietary abundance the amount of interstitium was greater, and the size of Leydig cells was larger than during drought. This latter finding is perhaps at variance with chronic testicular degeneration in domestic animal species – in which the interstitium/tubule ratio is increased as fibrous tissue replaces damaged tubules.

## **12. LESIONS OR DISEASES OF UNCERTAIN OR UNKNOWN AETIOLOGY**

### **Skin and subcutis**

**Calcinosis circumscripta** in a scrub (swamp) wallaby presented as chronic proliferative lesions on the plantar aspect of the feet between toes. The calcified area, which contained occasional bone-like spicules, was surrounded by a fibrous capsule.

### **Bone**

**Hypertrophic osteopathy** (HO), so-called Marie's disease, is described in a red kangaroo and a common wallaroo. The kangaroo was presented for examination because of lameness. There was bony thickening of all limbs due to periosteal new bone growth. There was no evidence of respiratory signs, and no cough was elicited, but 25 ml of pus – from which mixed coliforms were isolated – was aspirated from the thorax. Treatment with antibiotics was commenced, and within 6 weeks the lameness had resolved. The wallaroo was presented for examination with swelling of the forefeet. Clinical examination revealed thickening of the radius and ulna with taut overlying skin. A presumptive

diagnosis of HO was made. Subsequent necropsy findings included multiple caseous foci throughout the lungs associated with haemorrhage, consolidation, pleural adhesions and emphysema.

### **Teeth**

Unilateral failure of development of mandibular cheek teeth has been reported in a wild eastern grey kangaroo found dead. Premolars and molars were absent in one mandible, and there was corresponding lack of wear and abnormal elongation of the maxillary cheek teeth on the affected side. It was concluded that molar aplasia was congenital, and that an associated lumpy jaw lesion on the contralateral mandible may have been related to abnormal mastication. Maxillary molar progression, which is affected by abrasion, also seemed retarded on the affected side. Death was related to malnutrition, perhaps associated with the oral lesions, and a heavy parasite burden.

**Barber, D., J. Campbell, T. Luke et al. 2008. Unilateral failure of development of mandibular premolars in an eastern grey kangaroo (*Macropus giganteus*) and its effects on molar progression. Australian Veterinary Journal 86: 64-66.**

### **Liver**

Pronounced **amyloidosis** of unknown cause was observed in the liver of an eastern grey kangaroo. The liver was examined histologically because subcapsular haematomas approximately 10 cm in diameter were observed grossly.

An '**enigmatic**' **hepatopathy** of unknown but presumed parasitic cause was described in brush-tailed bettongs but apparently not in other marsupial species that were simultaneously housed in the same pens. Grossly, 12 animals had hepatomegaly characterised by large circular nodular lesions with dense fibrosis and variable calcification affecting one or more lobes. These lesions occupied up to half of the hepatic parenchyma.

Microscopically, in all but one case lesions were massive, and except in two cases all lesions were chronic. In these latter cases focal areas of hypercellularity consisted mainly of plasma cells, histiocytes, fibrocytes, lymphoid cells and occasional multinucleated giant cells. Centrally within these areas were the enigmatic, presumed parasitic bodies, some of which were surrounded by a rim of necrosis.

### **Circulatory system**

**Medial sclerosis and arterial calcification** were observed in more than 10% of 313 captive macropods, and these lesions showed considerable resemblance to those seen in humans and related to age. Although in this study the aetiology remained cryptic, a dietary cause was suspected – with cessation of the condition once diet was changed. Many species, but especially agile wallabies, were affected.

Grossly there was much variation although the aorta was consistently involved and had more severe changes than in other arteries. On cutting, the lesions were firm and gritty and affected vessels had little or no elasticity. Microscopically, lesions frequently presented as small foci or were linear along the long axis of the aorta. In some cases there was necrosis of smooth muscle and elastica.

Lesions suggestive of hypertension of unknown aetiology were evident in many adult western grey kangaroos from a single zoo in the USA. Clinical signs included vague nervous system deficits and blindness in a subset of affected animals. Thickening and hypertrophy of the media, and increased tortuosity of renal arterioles was characteristic.

**Kagan, R.A., M. Kinsell, K. Gloor et al. 2009. Morphologic evidence suggestive of hypertension in western grey kangaroos (*Macropus fuliginosus*). Veterinary Pathology 46: 977-984.**

### **Urinary system**

Although considered likely to be an inherited metabolic disease, **renal oxalosis** in the endangered Gilbert's potoroo is of uncertain causation. Clinically, glycolate in the urine of affected animals was greatly elevated. At necropsy, on removal of the capsule and on the cut surface the kidneys were rough and irregular, and microscopically – in animals that died – there were massive oxalate deposits present in renal tubules.

In a histological study of kidneys from 169 macropods of 16 species, **intranuclear inclusions** of unknown cause, but apparently of no clinical significance, were found in 9 animals from four species. Inclusions were seen only in captive animals, and were not found in joeys or adolescents. The inclusions, which were in the nuclei of proximal convoluted tubule cells of the renal cortex, were homogenous, eosinophilic, oval to spherical, and associated with margination of nuclear chromatin. Inclusions were approximately 5 µm in diameter and occupied about 75% of the nuclear area. Usually a single inclusion was present but occasionally two smaller inclusions were seen. Often all nuclei in affected tubules had inclusions while adjacent tubules had none. Histochemical examination of sections from an affected black-striped wallaby revealed that inclusions did not stain with Ziehl-Neelsen, von Kossa or PAS techniques. On electron microscopic examination the inclusions were seen to be homogeneously electron-dense. Viral particles or crystals were not seen.

Similar inclusions were observed in a bridled nailtail wallaby examined at the Australian Registry for Wildlife Health.

Cited early reports of **urolithiasis**, presumably of undetermined cause, in captive macropods include a calculus obstructing the neck of the urinary bladder in a red kangaroo, and renal calculi – one of which contained uric acid – in two wallabies. Bilateral renal calculi in a (presumed Tasmanian) pademelon measured 5mm x 5 mm, were yellow-grey and granular, and associated with pyelonephritis. They were composed mostly of an acidic, nitrogenous, phenolic compound.

Calculi composed of magnesium hydrogen phosphate are also reported in a captive young eastern grey kangaroo that was presented for examination because of dysuria. The animal's diet was quite varied and included dried cat food. Physical examination revealed gross distension of the bladder, and a 2 mm diameter calculus was found at the tip of the penile urethra. The animal died during attempts to pass a catheter and necropsy revealed two additional calculi 2 cm x 1 cm in the pelvic urethra. Associated changes included suppurative urethritis, periureteritis and bilateral pyelonephritis. Cause and pathogenesis of urolithiasis in this animal were not ascertained but dietary factors may have been involved.

#### **Multisystemic disease**

**Lysosomal storage disease** was suspected as the cause of illness in separate, young, eastern grey-, and red kangaroos. Cause of the lesions was not confirmed but possibilities included congenital disease or plant toxicosis. History, signs and gross findings in the two cases varied, but microscopically, in both cases the significant change was the widespread presence of foamy vacuolated macrophages, the contents of which was PAS positive.

A somewhat similar storage disorder was observed in a captive parma wallaby that showed neurological signs of sudden onset and was euthanased. No gross lesions were described but microscopically abundant brown, granular pigment – considered to be lipofuscin – was present in nerve cell bodies in grey matter of the brain and spinal cord. Suggested aetiologies were inherited lipofuscinosis and plant toxicosis, but in view of the history of similar clinical signs occurring in other wallabies confined to one enclosure, the latter possibility was preferred.

**Original Acknowledgements:** I am grateful to colleagues who kindly provided many of the images used in my presentation, and to Dr Larry Vogelneust for perusal of these notes prior to submission.