# Melioidosis in a koala

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In Australia, melioidosis has long been recognised as a serious and sometimes fatal disease of both domestic animals and man, especially in tropical areas (Thomas 1981; Guard *et al* 1984). In recent years, the causal organism, *Pseudomonas pseudomallei*, has been isolated from several Australian native species which were observed to be clinically ill (Thomas *et al* 1978, 1980; Saroja 1979). Described here is the isolation of *P. pseudomallei* from a lactating, free-living female koala (*Phascolarctos cinereus*) which died after being captured and released for routine survey purposes.

The subject was a 4-year-old female weighing 5.7 kg; a well furred 5-month-old infant koala was present in its pouch. It was captured on Magnetic Island in March 1989 by means of a lasso-type snare attached to an extendable aluminium pole. This procedure entailed the use of a red cloth, the movement of which induced the koala to climb backwards down the tree to a point where it was placed in a hessian sack (Cockram 1979). Little distress was apparent. Once restrained the koala was weighed, both eyes were examined for evidence of chlamydial infection, a blood sample was collected, a plastic identification tag was inserted into each ear lobe; then the koala was released back up its tree. Total restraint time was about 15 min.

Examination of both the mother and pouch young at time of capture revealed no illness. Approximately 40 h later, the koala was observed on the ground 100 m from the capture site. It died shortly afterwards and was submitted for necropsy examination. The pouch young was still alive; it was hand reared and fed at regular intervals on a milk supplement (Portagen, Bristol Meyers) for 36 h before it also died.

Necropsy of the adult koala, which was refrigerated for 24 h before examination, revealed about 4 ml of blood-stained fluid in the abdominal cavity, and pronounced ecchymotic and "paint brush" haemorrhages on most serous surfaces, especially in the thorax. Occasional ecchymoses were present on the endocardium and beneath the serosa of the kidney and urinary bladder. Many lymph nodes were dark due to congestion and haemorrhage.

Macroscopically, haemorrhages were confirmed beneath serosal surfaces, in smooth muscle of the urinary bladder and in the ovary. Marked congestion and some haemorrhages were present in the lung which contained bacteria unassociated with inflammatory infiltration. Congestion and haemorrhage were likewise noted in mycoardium, kidney, adrenal gland and lymph nodes, which also exhibited lymphoid depletion of follicles.

Major changes were evident in the spleen and liver. There was depletion of lymphocytes in the spleen with focal lympholysis and scattered foci of necrosis associated with fibrin accumulation, some neutrophilic infiltration, and early proliferation of histiocytes of bizarre shape. Similar necrotic foci, which however mostly lacked infiltrating neutrophils but in which the same histiocytic response was obvious, were frequent in the liver (Figures 1 and 2). A brown pigment, as previously described by Canfield *et al* (1986), was present in hepatocytes generally, and a particularly striking feature throughout the liver was the presence of distinctive eosinophilic intranuclear hepatocyte inclusions (Dickens 1975), which were unrelated to necrotic foci.

Only occasional bacterial colonies were seen associated with the necrotic foci in both liver and spleen. In sections stained by Gram's method, groups of small Gram negative bacilli appeared to be confined within the membranes of scattered degenerating cells. Similar intracellular Gram negative bacteria were present in occasional macrophages in the lung, unassociated with other lesions.

Fresh samples of liver and kidney were cultured aerobically. *Escherichia coli* and *P. pseudomallei* were isolated from both

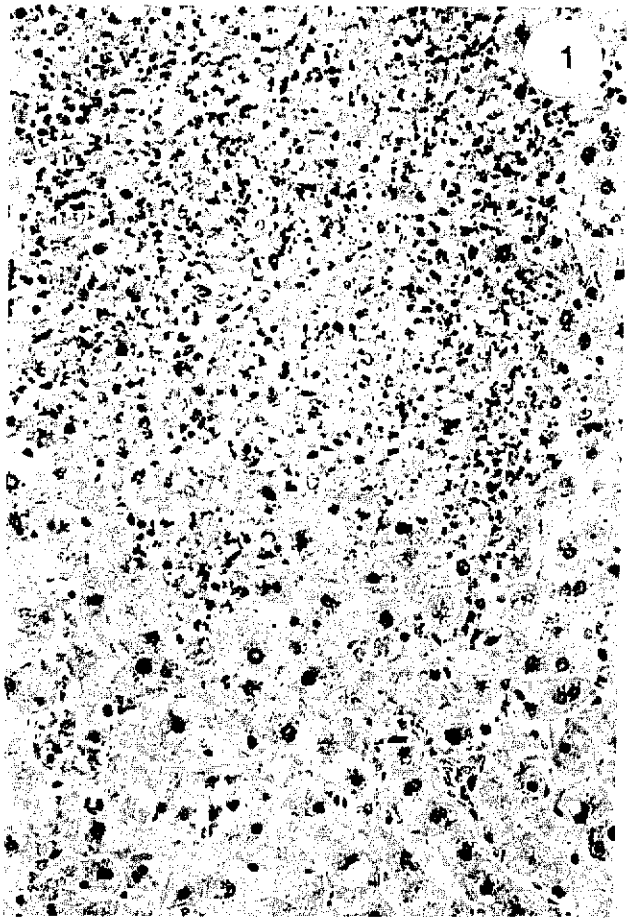


Figure 1. Necrotic foci in liver. Haematoxylin and eosin.

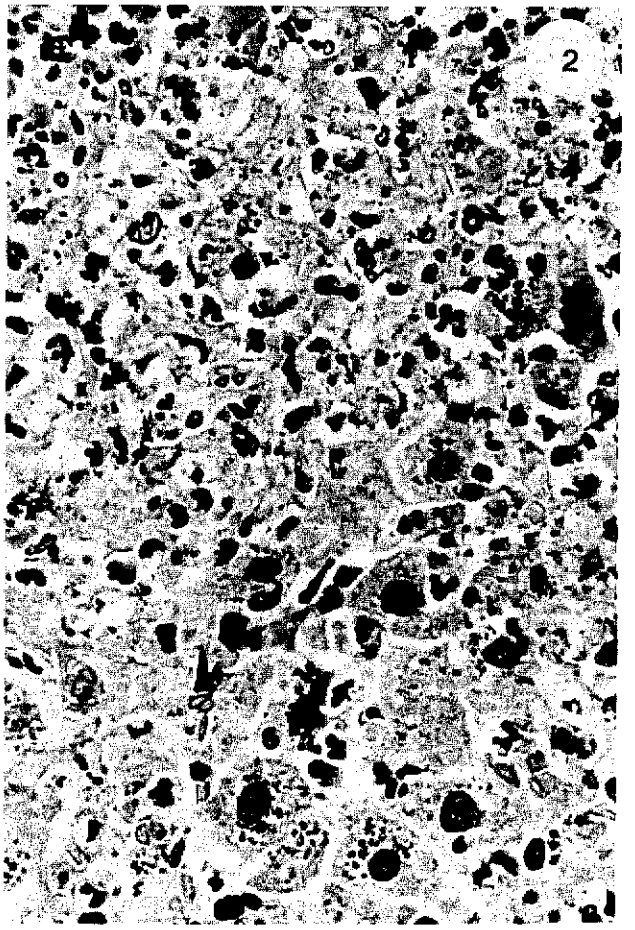


Figure 2. High magnification photomicrograph of periphery of lesion shown in Figure 1. Note necrotic area (top) with nuclear debris and presence of histiocytes of irregular shape. Granular pigment in remaining intact hepatocytes (below) is a feature of normal koala liver. Haematoxylin and eosin.

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organs. In addition, *Enterobacter cloacae* was isolated from the liver and both *Pseudomonas putida* and *Streptococcus* sp were isolated from the lung.

Necropsy of the infant koala revealed no significant changes. Microscopically, however, there was a diffuse interstitial pneumonia with proliferating histiocytes and scattered neutrophils in the distended interalveolar septa. In the liver, fatty change was pronounced and small foci of necrosis involving only several to 10 or so hepatocytes were common. The necrosis was accompanied by sinusoidal accumulation of some fibrin and a few neutrophils. Examination of Gram-stained sections of tissues from the infant koala did not reveal the presence of bacteria. Microbiological culture of tissues from the young koala was not done.

Serological examination of the adult koala for melioidosis using the complement fixation test (Thomas *et al* 1988) gave a negative result, but the serums of 8 of 50 (16%) free-living koalas in the same colony reacted at positive titre; the latter were apparently healthy.

So far as we are aware, *P. pseudomallei* infection of a koala has not been reported previously. The histological lesions we observed in the liver differ from abscess formation which was observed in most cases of melioidosis seen in domestic animals (Laws and Hall 1963).

The hepatic lesions in the adult koala closely resembled the so-called "paratyphoid nodules", which are a useful diagnostic feature in salmonellosis, but are also seen in infections with other bacteria such as *Listeria monocytogenes*, that proliferate intracellularly.

In experimental *P. pseudomallei* infection in goats, Narita *et al* (1982) found that, whereas 3 d after infection hepatic lesions were essentially small foci of necrosis with many neutrophils; 15 d after infection these focal lesions were distinctly granulomatous – thereby resembling the present case. Using electron microscopy, Narita *et al* (1982) confirmed the presence of *P. pseudomallei* in histiocytes.

An epidemiological aspect of this case that warrants comment is that, whereas *P. pseudomallei* is present in soil and water in particular areas, and infection in grazing animals seems often to be related to pasture-soil interactions during the "wet" season (Thomas *et al* 1979; Thomas and Forbes-Faulkner 1981), the diet of the koala is highly selective, being essentially restricted to the leaves of particular *Eucalyptus* species, together with the occasional but minor intake of soil and gravel (Bolliger 1962).

The fact that the present case occured in March after a wet season with higher-than-average rainfall is in agreement with previous findings (Thomas *et al* 1979). Presumably this koala became infected "between trees", by eating contaminated soil during its brief time on the ground. Possibly its susceptibility was heightened by the stress of lactation. Melioidosis in women has been associated with stress, trauma, diabetes and pregnancy (Guard *et al* 1984). Although stress-related factors associated with capture and handling may have exacerbated illness of the koala and hastened its death, the granulomatous component of hepatic lesions in the adult, and pulmonary lesions in the young koala, confirm that sickness in both animals preceded their capture and release.

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### References

Bolliger A (1962) – *Aust J Sci* **24:** 416
Canfield, PJ, Brown AS and Dickens RK (1986) – *Avian/Exotic Practice* **3:** 21
Cockram FA (1979) – Chlamydial keratoconjunctivitis of the koala, *Phascolarctos cinereus* (Goldfuss), MSc Thesis, Univ New England, Armidale, NSW, p 33
Dickens RK (1975) – *Aust Vet J* **51:** 459
Guard RW, Khafagi FA, Brigden MC and Ashdown LR (1984) – *Am J Trop Med Hyg* **33:** 467
Laws L and Hall WTK (1963) – *Qld J Agric Res* **20:** 499
Narita M, Loganathan P, Hussein A, Jumaluddin A and Joseph G (1982) – *Natl Inst Anim Health Q (Jpn)* **22:** 170
Saroja S (1979) – *Aust Vet J* **55:** 439
Thomas AD, Wilson AJ and Aubrey JN (1978) – *Aust Vet J* **54:** 306
Thomas AD, Forbes-Faulkner JC and Parker M (1979) – *Am J Epidemiol* **110:** 515
Thomas AD, Norton JH and Pott BW (1980) – *Aust Vet J* **56:** 192
Thomas AD (1981) – *Aust Vet J* **57:** 146
Thomas AD and Forbes-Faulkner JC (1981) – *Aust Vet J* **57:** 535
Thomas AD, Spinks GA, D'Arcy TL, Norton JH and Trueman KF (1988) – *Aust Vet J* **65:** 261

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# Use of a dietary supplement in koalas during systemic antibiotic treatment of chlamydial infection

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Infection with *Chlamydia psittaci* in the koala can cause blindness, pneumonia, infertility and urinary tract diseases and has been described by many workers (Cockram and Jackson 1974; Dickens 1976; Cockram 1978; Brown *et al* 1980; Brown and Grice 1984). A survey of both captive and wild koalas throughout eastern Australia from 1979 to 1985 indicated that a large proportion of koalas sampled was affected by *C. psittaci* (Brown and Carrick 1985).

*Ad hoc* attempts to treat chlamydial infections in koalas with antibiotics usually failed, although Carrick and Wood (1986) found that administration of dietary supplements enabled the majority of koalas treated with oxytetracycline by injection to survive.

Nevertheless, Brown (1987) reported that systemic antibiotic therapy for chlamydial infections in koalas, using either erythromycin or oxytetracylcine, was invariably fatal. The koalas showed rapid loss of body weight and died, although there was a marked reduction in signs of chlamydiosis during the course of the treatments.

As suggested by Carrick and Wood (1986), supplementary feeding using soya-based infant feeding preparation has become a common management practice for captive koalas in many zoos, including Lone Pine Koala Sanctuary in Australia, and Saitama Children's Zoo and Awaji Farm Park in Japan. Handasysde *et al* (1988) have also since recommended such supplementary feeding for captive koalas showing consistent loss of weight. We have conducted a controlled experiment to evaluate the efficacy of such dietary supplementation during systemic antibiotic therapy in koalas.

Eight koalas showing kerato-conjunctivitis and positive antichlamyidal CF titre were studied. These koalas were presented to the Koala Study Program at the University of Queensland between 1 September 1987 and 31 October 1987, and housed in the Koala Study Program's facilities at the University of Queensland Veterinary Science Farm. Forked timber poles and linking horizontal branches were placed within these holding facilities to provide suitable artificial trees on which each koala could

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