Cystic echinococcosis in a wild population of the brush-tailed rock-wallaby (*Petrogale penicillata*), a threatened macropodid

T. S. BARNES[1]*, A. W. GOLDIZEN[2], J. M. MORTON[1] *and* G. T. COLEMAN[1]

[1] *School of Veterinary Science and* [2] *School of Integrative Biology, University of Queensland, Qld. 4072, Australia*

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SUMMARY

Infection of small macropodids with the larval stage of *Echinococcus granulosus* can cause fatalities as well as significant pulmonary impairment and other adverse sequelae. The brush-tailed rock-wallaby (*Petrogale penicillata*) is a small macropodid listed as vulnerable on the IUCN's *Red List of Threatened Species*. This study used radiographic techniques to determine the prevalence and severity of pulmonary hydatid infection and growth rates of hydatid cysts in a wild population of this macropodid. The overall prevalence was 15·3% (9/59 animals) with 20·0% (8/40 animals) of adults infected. During the study period, the death of at least 1 infected animal was directly attributed to pulmonary hydatidosis. Rapid cyst growth occurred in some animals (up to 43% increase in cyst volume in 3 months). Cyst volume reduced lung capacity by up to 17%. Secondary pulmonary changes were uncommon but, in 1 animal, resulted in reduction in lung capacity by approximately 50%. Infection was associated with a higher blood urea concentration, but no significant differences in other blood variables were detected. These results indicate that hydatid infection may be a significant risk to threatened populations of small macropodids and should be addressed in conservation management plans for these animals.

Key words: *Echinococcus granulosus*, hydatid, marsupial, macropodid, brush-tailed rock-wallaby, *Petrogale penicillata*, conservation, Australia.

INTRODUCTION

Human colonization and introduction of domestic animals have resulted in widespread dissemination of many pathogens. In some cases, such agents have proven to be highly pathogenic, and their impact on wildlife populations has been devastating (Daszak *et al*. 2000). Threatened species are particularly susceptible to the effects of generalist parasites (those with a broad host range), because reservoir hosts can maintain infection pressure (McCallum and Dobson, 1995; Riordan *et al*. 2006). *Echinococcus granulosus*, the causative agent of hydatid disease or cystic echinococcosis, is a generalist parasite believed to have been introduced to Australia with European settlement (Jenkins and Macpherson, 2003). Australian isolates of this cestode, examined to date, have been genetically indistinguishable from the common 'sheep strain' (Hope *et al*. 1992). In addition to the widely recognized domestic cycle between sheep and dogs, a sylvatic cycle exists in Australia that utilizes a variety of macropodid species and feral pigs (*Sus scrofa*) as common intermediate hosts (Kumaratilake and Thompson, 1982), and wild dogs (dingoes, *Canis lupus dingo*, and their hybrids) and foxes (*Vulpes vulpes*) as definitive hosts (Durie and Riek, 1952; Jenkins and Morris, 1991). The two cycles are not mutually exclusive; sheep can act as intermediate hosts for wild dogs and foxes, and macropodids and feral pigs can act as intermediate hosts for dogs. Hydatid cysts occur most commonly in the lungs of macropodid hosts; only 0·5% of cysts were found outside of the thoracic cavity in 71 naturally infected eastern grey kangaroos and wallaroos and 5% of cysts from 11 experimentally infected tammar wallabies (Barnes *et al*. 2007*a*, *b*).

Four Australian macropodids have become extinct since European settlement and a further 3 small macropodids are listed on the IUCN Red List of Threatened Species (2006); the bridled nailtail wallaby (*Onychogalea fraenata*) and the Proserpine rock-wallaby (*Petrogale persephone*) are listed as 'endangered' and the brush-tailed rock-wallaby (*P. penicillata*) as 'vulnerable'. Several other species have distributions that have contracted over the last 2 centuries, leading to concerns for their long-term survival (Burbidge and McKenzie, 1989). Several threatening processes have been implicated in the decline of these species: predation by introduced carnivores, competition with introduced herbivores

* Corresponding author: School of Veterinary Science, University of Queensland, Qld. 4072, Australia. Tel: +61 7 3365 3203. Fax: +61 7 3365 1255. E-mail: t.barnes@uq.edu.au

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and habitat loss and fragmentation (Strahan, 1995; Lundie-Jenkins and Lowry, 2005; Menkhorst and Jarman, 2005). However, diseases, including those associated with the parasites *E. granulosus* and *Toxoplasma gondii*, have also been suggested as factors contributing to such declines (Eldridge, 1997; Nolan and Johnson, 2001; Lundie-Jenkins and Lowry, 2005). Unfortunately, studies of parasites and other pathogens of endangered species have generally only been undertaken following epidemics of mortalities which have devastated small populations (Cleaveland *et al*. 2001). Recovery plans that include captive breeding, translocation and re-introduction programmes have been initiated for several macropodid species, including the brush-tailed rock-wallaby (Nolan and Johnson, 2001; Lundie-Jenkins and Lowry, 2005; Menkhorst and Jarman, 2005) but, clearly, a thorough understanding of the epidemiology of significant diseases and how they affect populations of these animals would enable better outcomes from such strategies.

The brush-tailed rock-wallaby is a medium-sized macropodid. Although once abundant and widespread throughout the mountainous country of south east Australia, the species is now nearly extinct in the state of Victoria and populations are declining in the states of New South Wales and Queensland (Clancy and Close, 1997; Dovey *et al*. 1997). In addition to its IUCN listing as vulnerable, the species is listed in Australian legislation as vulnerable nationally, threatened in Victoria, endangered in New South Wales and the Australian Capital Territory and vulnerable in Queensland (Menkhorst and Jarman, 2005). The brush-tailed rock-wallaby is found in small colonies, usually consisting of less than 30 individuals (Jarman and Bayne, 1997). In this prospective, longitudinal study of hydatid infection in wild macropodids, we determined the prevalence of pulmonary *E. granulosus* infection in 3 neighbouring colonies of brush-tailed rock-wallabies by chest radiography, a technique validated during experimental infection of tammar wallabies (Barnes *et al*. 2007*a*). We estimated the percentage loss of lung capacity in infected animals and monitored the rate of cyst growth in some individuals. We aimed to determine the effect of *E. granulosus* infection on body condition indices and blood variables and the potential significance of infection by this parasite at both the individual and population levels. Implications for conservation management plans for the brush-tailed rock-wallaby are considered.

MATERIALS AND METHODS

Study site

The Hurdle Creek, Farm Creek and Farm Creek East brush-tailed rock-wallaby colonies, located on private properties above the southern cliffs of Hurdle Creek valley and the southern side of Farm Creek valley, Mount Colliery, Queensland (28°17′S, 152°19′E, altitude range: 850–1050 m) have been the focus of behavioural, mark-recapture and genetic studies (Laws and Goldizen, 2003; Hazlitt *et al*. 2004) since late 2000. The Hurdle Creek colony is known to comprise 35–40 animals, including juveniles, at any one time (Laws and Goldizen, 2003). The Farm Creek and Farm Creek East colonies have been less well studied, but their total population sizes were thought to be smaller (Hazlitt *et al*. 2006).

The study site is in a summer rainfall area and therefore experiences relatively warm, wet summers and cool, dry winters. The average rainfall at Tannymorel, approximately 7 km from the study site, is 483 mm from October to March, but only 200 mm from April to September. Mean maximum and minimum daily temperatures at Warwick, approximately 40 km north-west of the study site, are 29·9 and 16·6 °C in summer and 18·9 and 3·5 °C in winter (Bureau of Meteorology, Australian Government).

History of cystic echinococcosis in the study area

Two brush-tailed rock-wallaby fatalities related to hydatid infection occurred in the study population and a neighbouring population before this study began. One death occurred during trapping, and *post-mortem* examination revealed that this wallaby had multiple *E. granulosus* cysts in one lung (A. W. Goldizen, unpublished data). At the time the current study commenced, more than 800 examinations of animals had been conducted at the site and no other adverse reactions to handling had been recorded. Another wallaby from a neighbouring colony had died 2 years earlier when pursued uphill for less than 10 m. This animal had 2 large cysts in the right lung and the remaining tissue from this lung was severely compromised (G. Mifsud, personal communication).

Study design

In this prospective, longitudinal study, brush-tailed rock-wallabies were caught over 5 trapping periods: (1) November 2004 (Hurdle Creek), (2) January/February 2005 (Hurdle Creek and Farm Creek East), (3) April/May 2005 (Hurdle Creek and Farm Creek East), (4) July/August 2005 (all colonies) and (5) November 2005 (all colonies but only for follow-up radiographs of animals infected with *E. granulosus*). Each trapping period was 2–4 weeks in duration. Wallabies were trapped along the tops of cliffs in metal treadle traps, baited with apple and sweet potato and lined with foam and shade cloth. They were caught after sunset as they emerged above the cliffs to feed in the grassland and mixed eucalypt forest

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(Laws and Goldizen, 2003). All traps were checked within 4 h of setting and, on some occasions, traps were reset and checked twice per night. On the first occasion that each wallaby was trapped during the course of the study, it was anaesthetized and chest radiographs taken. Wallabies recently emerged from the pouch were not radiographed, as they were very unlikely to have radiographically visible hydatid cysts. Only those animals previously detected as being infected with *E. granulosus* were radiographed again, during trapping period 5. On the first occasion, a wallaby was caught during each trapping period, body condition and reproductive data were collected and a blood sample taken.

Identification and external examination

A microchip (LifeChip/Digivet.com Pty. Ltd, Baulkham Hills, NSW, Australia) was injected subcutaneously into each trapped wallaby. Colony, gender, age class (either adult or subadult), weight to nearest 100 g and left hind foot lengths to nearest 0·1 mm were recorded for all individuals trapped in each trapping period. Females were classed as adults if they had a pouch young or one or more teats had been used (Poole and Catling, 1974). Males were classed as adults if they were >5 kg and had descended testes. The condition index was estimated as the residuals from a regression of $\log_e$ (weight) on $\log_e$ (hind foot length), determined separately for each age class and sex (Pople *et al*. 2001). Foot length of pouch young was recorded and pouch young weight was estimated using a weight *versus* foot size curve for pouch young previously established for the yellow-footed rock-wallaby (*P. xanthopus*) by Sharp (2002) and subsequently validated for the brush-tailed rock-wallaby (Wynd *et al*. 2006). The weight of adult females with pouch young was calculated as the combined weight minus the weight of pouch young.

Radiography

For radiographic examinations, wallabies were transported in hessian sacks back to the field camp, a maximum 30 min walk and/or drive from all trap sites. Each wallaby was anaesthetized using 2–3% isoflurane (Attane, Pharmtech) in oxygen, with a flow rate of 500 ml/kg/min, administered by a closely fitting face mask and valveless Bain's paediatric circuit suitable for the anaesthesia of small dogs (Seymour and Gleed, 1999). Wallabies remained in the sacks during induction to minimize handling stress and anaesthesia was maintained with 2–2·5% isoflurane for the required duration. Oxygen was then administered until voluntary movement returned and animals were held in hessian sacks for $\geqslant 30$ min prior to release at the site where they had been trapped.

Left- and right-lateral and dorso-ventral radiographs of each wallaby's chest were taken using a portable veterinary x-ray unit (Aloma, MT 40), calcium tungstate intensifying screens and Fuji Super RX film. Exposures were as follows: animals <4 kg – lateral views, 50 kV/40 mA/0·04 sec, dorso-ventral views, 50 kV/40 mA/0·06 sec; animals >4 kg – lateral views, 60 kV/40 mA/0·04 sec, dorso-ventral views 70 kV/25 mA/0·04 sec. Animals were held in position with foam and sand-bag positioning aids. Radiographs were developed in an automatic processing unit (Konica SRX 701) at Warwick Hospital.

Radiographs were interpreted independently by the first author and an experienced veterinary radiologist in the University of Queensland. Where possible, the diameters of all opacities indicative of *E. granulosus* cysts were measured in 2 planes using the radiographic view that provided the clearest visualization of the lesion. For each cyst, the same view was used to measure cyst diameters on the 'follow-up' radiograph. The approximate volume was calculated using the average diameter and assuming that the cyst was spherical. Total lung volume of a 5·2 kg tammar wallaby (*Macropus eugenii*), a species similar in weight and body structure to the brush-tailed rock-wallaby, has been estimated at 240 cm^3 (Barnes *et al*. 2007*a*). Thus the percentage loss of lung capacity due to the presence of hydatid cyst(s) was estimated as (total cyst volume/240)*100.

Collection of blood samples

Blood samples (2–4 ml) were taken from either the lateral coccygeal vein or the jugular vein. One ml of blood was added to an ethylenediaminetetraacetic acid (EDTA) tube and stored at 4 °C for haematological examination; blood smears were made within 2 h of collection. The remaining blood from each sample was transferred to a clot-activating tube and the serum separated by centrifugation (1500 ***g*** at 4 °C for 10 min), and stored at −20 °C prior to biochemical analysis.

Haematology and biochemistry

Haematological analyses used an Abaxis Vetscan HMT analyser (Union City, California) for the following haematological variables: haemoglobin (Hb), red blood cell count (RCC), packed cell volume (PCV) and white blood cell count. Differential counts of white cells ($n=100$) were made on smears fixed and stained using Wright's methanol-based stain. The smears were examined under light microscopy (Olympus BH2) at 400× magnification. Sera were analysed with an Olympus AU400 biochemistry analyser for aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl

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transferase (GGT), total protein, albumin, globulin and urea.

Post-mortem examination

One wallaby died during the course of the study and was necropsied within 2 h of death. Samples of grossly abnormal tissue were fixed in 10% buffered formalin and later processed using standard histological methods (Lillie, 1954).

Statistical analyses

All statistical analyses were performed using Stata v.9 (StataCorp, College Station, Texas USA, 2006). To assess the effects of age class, sex and colony on the likelihood of animals being diagnosed with cystic echinococcosis, univariable logistic regression was undertaken with each exposure variable fitted separately and assessed in comparison to the null model using the likelihood ratio test. Associations for each colony were also compared with the reference colony (Hurdle Creek) using Wald tests. The exposure variables were also fitted in a multivariable model and the odds ratios and *P*-values compared with those obtained in the univariable models.

Associations of hydatid infection with selected blood variables and condition indices were assessed using generalised estimating equations to account for dependence of repeated measurements within wallabies. Only data from the Hurdle Creek colony were used in analyses as data from Farm Creek were only collected once and the sample size from Farm Creek East was very small (13 animal-examinations involving 8 animals). Normally distributed residuals, identity link functions, exchangeable correlation structures and robust standard errors were used and time was fitted as a categorical variable. Only those associations considered biologically plausible *a priori* or previously identified (Barnes *et al*. 2007*a*) were selected for these analyses. Thus associations were assessed between cystic echinococcosis as the putative explanatory variable and eosinophil count, white cell count, urea, AST, ALP, GGT, protein, albumin, globulin as outcome variables. Blood variables were log transformed when required to reduce skewness. Associations were modelled with adjustment for age class, sex, trapping period and/or method of restraint if these had been previously associated with the outcome variable (Barnes *et al*. 2008).

RESULTS

During the course of the study 59 animals were radiographed, including 39 animals from the Hurdle Creek colony, 7 animals from the Farm Creek colony, and 13 animals from the Farm Creek East colony. Repeat radiographs were taken of 5 of the 9 animals diagnosed with *E. granulosus* infection. Blood samples and condition data were collected at 132 animal examinations; of these, 107 examinations of 39 individual animals at Hurdle Creek were used for statistical analyses of associations of cystic echinococcosis with selected blood variables and condition indices. Rainfall throughout the study period was below average (Bureau of Meteorology, Australian Government).

Radiographic changes consistent with hydatid infection were found in 9 of the 59 animals examined, equating to 15·3%, with 20·0% (8/40) of adults and 5·3% (1/19) of subadults having been infected. Details of these animals and their radiographic lesions are given in Table 1. No significant associations were detected between animal age-class, sex or colony and hydatid infection ($P>0{\cdot}1$, Table 2). However, relative to adults, the observed odds ratio for subadults was 0·22 (95% CI 0·03, 1·92) and relative to animals at Hurdle Creek, those at Farm Creek had an observed odds ratio of 3·89 (95% CI 0·81, 18·65; Wald $P=0{\cdot}08$). Observed odds ratios and *P*-values were similar for all exposure variables in the multivariable model with age-class, sex and colony fitted, so univariable results are reported.

At their first radiographic examination, 5 wallabies each had 1 radiographically detectable cyst, 3 had 2 cysts and 1 had 2 clearly defined cysts and an abnormal area of lung suggestive of a third cyst (not included in measurements). Initial radiographs of these wallabies showed losses of lung volume due to cysts of 7·2 to 28·0 cm^3 (Table 1). Radiographic changes in the lung tissue surrounding cysts were also evident in 1 wallaby, in which the cyst was located cranially within the right lung and all lung tissue caudal to the cyst showed a pronounced alveolar pattern consistent with intrapulmonary bronchopneumonia, haemorrhage or oedema.

Five of the 9 infected animals were radiographed again at 3 or 6 months after their initial radiograph (Table 1). Estimated losses of lung capacity due to hydatid cysts were calculated on both occasions for 4 of these animals. Losses were estimated at 3·0–11·7% based on the initial radiographs and increased to 5·0–17·3% on the 'follow-up' radiographs. This represented an increase in cyst volume of up to 43% over a 3-month period and 68% over 6 months. The second radiograph of the fifth animal revealed an ill-defined area with an alveolar pattern where the larger cyst was seen in the initial radiograph. Three of the infected animals were not re-trapped during the final field trip. The wallaby with evidence of severe surrounding lung pathology, died during trapping period 5, one year after initial radiographic diagnosis of cystic echinococcosis. At the time, it was in good body condition with a condition index of 0·074 (positive values are above average). *Post-mortem* examination revealed one *E. granulosus* cyst,

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Table 1. Details of nine wild brush-tailed rock-wallabies showing radiographic evidence of hydatid infection

(Animals were initially radiographed at 1 of 4 trapping periods from November 2004 to August 2005 at Hurdle Creek, south-east Queensland. Repeat radiographs were taken of 5 animals, and 1 animal was necropsied in November 2005. Results from these repeat measurements are shown in italics.)

Sex	Age class	Trapping period	Weight (kg)	No. cysts	Cyst 1 vol (cm^3)	Cyst 2 vol (cm^3)	Total cyst volume (cm^3)	% Estimated loss lung volume[a] (% increase in cyst volume)
F	Adult	4	5·0	2	10·9	17·2	28·1	11·7
	Adult	*5*	*4·9*		*18·0*	*23·4*	*41·4*	*17·3* (*43*)
F	Subadult	4	4·8	2	25·5	0·4	25·9	10·8
	Adult	*5*	*5·2*	—[b]	*Not defined*	*0·5*	*Not calculated*	*Not calculated*
F	Adult	4	5·3	2/3[c]	18·8	0·4	19·2	8·0
	Adult	*5*	*5·9*		*24·4*	*1·4*	*25·8*	*10·8* (*34*)
M	Adult	4	5·7	1	18·8		18·8	7·8
F	Adult	2	5·0	1	17·2		17·2	7·2
F[d]	Adult	1	5·3	1	15·6		15·6	6·5
F	Adult	4	5·5	2	12·8	0·6	13·4	5·6
	Adult	*5*	*5·5*		*14·1*	*2·0*	*16·1*	*6·7* (*20*)
F	Adult	1	6·2	1	7·7		7·7	3·2
	Adult	*5*	*6·1*		*9·9*[e]		*9·9*	*4·1* (*29*)
M	Adult	3	7·1	1	7·2		7·2	3·0
	Adult	*5*	*7·3*		*12·1*		*12·1*	*5·0* (*68*)

[a] Percentage loss of lung volume is based on an estimate of total lung volume of 240 cm^3 in a 5·2 kg tammar wallaby (Barnes *et al*. 2007*a*). Consequently this value will be an overestimate for animals weighing more than 5·2 kg and an underestimate for those weighing less than 5·2 kg.
[b] Unknown, the larger cyst, seen on the previous radiograph, could not be clearly visualized.
[c] Radiographs showed an abnormal area suggestive of a third cyst but as it was not clearly defined it was not included in the measurements.
[d] Animal had been regularly trapped during previous studies, but was not re-trapped following diagnosis of infection.
[e] Cyst volume is estimated from post-mortem measurements. Animal died during trapping.

Table 2. Univariable associations between selected putative risk factors and hydatid infection using data from wild brush-tailed rock-wallabies trapped over four trapping periods from November 2004 to August 2005 at Hurdle Creek, south east Queensland

Exposure variable	No. examined	% infected	Odds Ratio (95% CI)	*P*-value
Age-class				0·112
Adult	40	20·0	1·00[a]	
Subadult	19	5·3	0·22 (0·03, 1·92)	
Sex				0·348
Female	38	18·4	1·00[a]	
Male	21	9·5	0·47 (0·09, 2·48)	
Colony				0·245
Hurdle Ck	39	10·3	1·00[a]	
Farm Ck East	7	14·3	1·46 (0·14, 15·39)	
Farm Ck	13	30·7	3·89 (0·81, 18·65)	

[a] Reference group.

irregular in shape, occupying the middle of the right lung. The estimated cyst volume of 9·9 cm^3 was 29% larger than that at the previous radiographic estimate 12 months earlier. Most of the cyst fluid had been resorbed, the germinal membrane was involuted, and there were signs of caseation. The cyst contained protoscoleces, but their viability was not assessed. The right, caudal lobe was firm, congested and oedematous. This was consistent with the alveolar pattern recorded radiographically in the previous year and suggested that the impact of hydatid infection on lung capacity in this animal was greater

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than that expected based solely on the physical size of the cyst.

Cystic echinococcosis was associated with a higher blood urea concentration (estimated increase with infection 1·03 mmol/l; CI 0·40, 1·67, $P=0·001$, crude mean value 10·0 mmol/l) but not with significant changes in any of the other blood variables ($P>0·098$), although point estimates were imprecise. The estimate of association between hydatid infection (as the explanatory variable) and condition index was imprecise, such that actual effects are uncertain.

DISCUSSION

Echinococcus granulosus infection was common in these colonies of wild brush-tailed rock-wallabies and appeared to have contributed to the death of an animal during the course of the study, in addition to multiple fatalities recorded before the study commenced. These fatalities were associated with loss in functionality of all or most of one lung. Cystic echinococcosis has been reported previously to cause mortality in small macropodids (Johnson *et al*. 1998), but the prevalence of infection and consequences of less severe infections are not known. Cysts develop most commonly in the lungs of macropodid hosts (Thompson *et al*. 1988; Barnes *et al*. 2007*b*). The loss of lung capacity, of up to 17·3% in surviving animals and up to 50% in the fatalities (1 during and 2 prior to the study), would likely be of little consequence for animals at rest, but could severely affect their ability to withstand stress or exertion, resulting in an increased susceptibility to predation.

The rate of cyst growth in an experimentally infected group of tammar wallabies was very rapid compared with that in the parasite-adapted intermediate host, the sheep, and marked loss of lung capacity was common in these tammars within a year of infection (Barnes *et al*. 2007*a*). In 4 of the 5 rock-wallabies that were re-radiographed in the present study, cysts had growth rates of up to 43% after 3 months and 68% after 6 months, suggesting that rapid growth is also a feature of natural infection in brush-tailed rock-wallabies. These growth rates indicate that the infection, particularly with multiple cysts, is likely to significantly compromise pulmonary function within a few years. Only 1 cyst in 1 wallaby was no longer detectable on follow-up radiography, and all other cysts increased in size. This indicates that cyst degeneration occurs in only a small proportion of infected animals, at least within the 12 month time-frame of this study. These findings concur with those from experimental infection of tammar wallabies, where cyst degeneration was recorded in only 18·2% (2/11) animals over a 9 to 16-month period (Barnes *et al*. 2007*a*).

Urea concentrations of infected brush-tailed rock-wallabies were approximately 10% higher than those of uninfected ones. In experimentally infected tammar wallabies, a 25% elevation in blood urea concentration was also recorded at 4 months, but not 8 months, following the oral administration of *E. granulosus* eggs (Barnes *et al*. 2007*a*). The biological significance of this finding remains unclear. Other changes to blood variables recorded in experimentally infected tammar wallabies were an increase in globulin at 4 months and a decrease in total white cell count at 8 months following infection, but neither change was detected in infected brush-tailed rock-wallabies. However, the duration of infection in the latter species was not known, and transient changes are unlikely to have been detected in these analyses. In the experimental infection of tammar wallabies (Barnes *et al*. 2007*a*), animals in poorer condition and/or with blood values indicative of lower protein status were more susceptible to cyst development following the administration of *E. granulosus* eggs. Given the low rainfall during the course of the study of brush-tailed rock-wallabies, and the years of relative drought leading up to the study period (Wynd *et al*. 2006), it is possible that the wallabies were on a low plane of nutrition and thus at increased risk of becoming infected and/or rapid development of cysts. The low rainfall is unlikely to have caused reduced infection pressure, as it remained above that cited as favourable for parasite survival (i.e. >25 mm per month for at least 6 months of the year; Gemmell, 1958) for all but 1 year from 1995–2005.

This study is the first to estimate the prevalence of cystic echinococcosis in live, wild macropodids, based on sampling the majority of the population. Previous records of prevalence of *E. granulosus* infection have relied upon necropsy (Beveridge *et al*. 1989; Jenkins and Macpherson, 2003; Banks *et al*. 2006; Barnes *et al*. 2007*b*), with the exception of a serological study that relied upon an assay that has not been validated in macropodids (Turni and Smales, 2001). Prevalences of up to 57% have been demonstrated (Jenkins and Morris, 2003). However, these studies sampled only a small proportion of the macropodid populations. In contrast, previous estimates of the size of the Hurdle Creek colony (Laws and Goldizen, 2003) suggest that almost all the colony's animals were radiographed in the current study. The prevalence of 15·3% in the study population is higher than reported previously from small studies in which rock-wallabies were necropsied; 10% prevalence was reported in 13 Godman's rock-wallabies (*Petrogale godmani*) and 5% prevalence in 20 Proserpine rock-wallabies (*P. persephone*) sampled in north Queensland (Beveridge *et al*. 1989; Begg *et al*. 1995).

Interestingly, the present results suggest that prevalence may vary substantially between colonies. Such clustering of infection on a small scale is not surprising, given that it has been demonstrated at a property level in eastern grey kangaroos

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(*M. giganteus*), macropodids that have a much larger 'home range' than the brush-tailed rock-wallaby (Barnes *et al.* 2007*b*). No colony-level risk factors were investigated during this study, but it is possible that dingo and/or fox density may be contributing factors.

At this study site, the transmission of *E. granulosus* is almost certainly restricted to the sylvatic cycle; domestic stock are unlikely to act as reservoir hosts as sheep are not grazed in the area and cattle, although present, are dead end hosts for the parasite (Kumaratilake and Thompson, 1982). However, other macropodid species, such as wallaroos (*Macropus robustus*) and red-necked wallabies (*M. rufogriseus*), are found at and around the study area and may act as reservoirs. All potential definitive hosts for the parasite (feral dogs, dingoes and foxes), have been recorded at and around the Hurdle Creek study site. The likelihood of transmission of *E. granulosus* to these hosts may be greater than in the domestic cycle, as infection of the intermediate host is associated with mortalities or reduced fitness in some cases, thereby increasing the likelihood of cyst ingestion through scavenging and predation by carnivores. Using methods described by Jenkins *et al.* (2000), antigens from *E. granulosus* (*i.e.*, coproantigens) were detected in a faecal sample from a dingo collected at Farm Creek, supporting the involvement of dingoes in the epidemiology of *E. granulosus* in this area (T. S. Barnes and D. J. Jenkins, unpublished data). As farm dogs were not kept on the properties but only visited during stock inspection, their contribution as definitive hosts is likely to have been insignificant. Unfortunately, faecal samples from these dogs were not available for coproantigen testing to test this proposal.

The imprecise estimate of association of *E. granulosus* infection and condition index does not allow us to conclude whether or not this infection is detrimental to body condition of the rock-wallabies. In addition, the cross-sectional study design may have resulted in a selection bias, such that only relatively healthy animals that were actively foraging were trapped, whereas those that were unhealthy or had died within the population were not trapped and their infection status was not determined. This situation could have resulted in an underestimation of the potential significance of infection to the population in a manner similar to the healthy worker effect (Wen *et al.* 1983). These problems reflect the difficulties outlined by McCallum (1994) in quantifying the pathogenicity and impact of parasites on host populations. He suggested that experimental manipulation of parasite burdens is required to provide definitive answers, but this was beyond the scope of a study on a threatened species.

The findings of a high prevalence of cystic echinococcosis, rapid cyst growth and the association between infection and fatalities at this study site suggest that *E. granulosus* is an essential consideration for conservation management plans for threatened small macropodids. This view is reinforced by the findings from experimental infection in tammar wallaby, in which 65·3% (7/11) of infected animals either died as a result of infection or were euthanased on welfare grounds within 16 months of infection (Barnes *et al.* 2007*a*). Efforts should focus on effective canid control. Also, there is a recombinant vaccine, EG95, that is known to give effective protection to sheep (Lightowlers *et al.* 1999). Current work is exploring the efficacy of this vaccine to protect macropodids against cystic echinococcosis, in the hope that captive release programs may incorporate pre-release vaccination to provide protection against *E. granulosus*.

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