

## PARASITES OF THE BRUSH-TAILED ROCK-WALLABY (*PETROGALE PENICILLATA*)

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**ABSTRACT:** The brush-tailed rock-wallaby (*Petrogale penicillata*) is listed as vulnerable on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. Parasitic diseases have been proposed as possible contributing factors to the decline of the species, but very little is known about the effects of parasites on this host. This study determined the antibody prevalence of the protist *Toxoplasma gondii* in a wild brush-tailed rock-wallaby population from three neighboring colonies in southeast Queensland, Australia. Fecal egg and oocyst count, tick count, severity of skin rash, and presence of lice and microfilariae were also monitored during four or five trapping periods over 1 yr. Antibodies against *T. gondii* were detected in 5% of animals (3/64). Fecal egg and oocyst counts were highly variable, but fecal egg counts were lower in subadult animals relative to adults. Neither fecal egg count nor oocyst count was associated with variation in blood variables or condition index, but a negative association between fecal egg count and oocyst count was observed. Microfilariae (*Breinvia spelaea*), lice (*Heterodoxus octoseriatus*), and skin lesions were seen more frequently during the November trapping period. A mite, *Thadeua* sp., was more likely to be detected in these skin lesions than in skin of unaffected wallabies. Tick (*Ixodes holocyclus* and *Haemaphysalis bancrofti*) counts also varied between trapping periods and were lowest in the April/May trapping period. This study provides the most detailed account to date of parasite burdens in a vulnerable macropodid, but no clear evidence emerged linking parasites to adverse impact on the host.

**Key words:** Brush-tailed rock-wallaby, coccidia, ectoparasite, fecal egg count, macropodid, *Petrogale penicillata*, *Toxoplasma gondii*.

### INTRODUCTION

Parasites can cause mortality, reduce reproductive success, and/or change host behavior to such an extent that they regulate the host population (Hudson, 1986; Tompkins and Begon, 1999). Little is known about the impact of parasites on macropodids. The nematode *Globocephaloides trifidospicularis* can cause significant mortality and morbidity in young eastern grey kangaroos (*Macropus giganteus*; Arundel et al., 1990), and severe coccidiosis may occur in eastern grey kangaroos in captivity and in flooded habitats (Winter, 1959; Barker and Harrigan, 1972). Macropodids in Australia are also susceptible to two introduced generalist parasites, *Echinococcus granulosus* and *Toxoplasma gondii* (Obendorf and Munday, 1983; Johnson et al., 1988). The impact of the former on wild populations of the brush-tailed rock-wallaby (*Petrogale penicillata*) has been described previously

(Barnes et al., 2008b). Clinical disease associated with *Toxoplasma* infection is well recognized in captive macropodids (Boorman et al., 1977; Patton et al., 1986) but has only been reported once in wild animals (Obendorf and Munday, 1983).

Although the abundance of the brush-tailed rock-wallaby varies across regions in Australia, it is listed on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species as vulnerable (IUCN, 2006). Several threatening processes have been identified, including diseases caused by parasites, and the species is the subject of a national recovery plan (Strahan, 1995; Eldridge and Close, 1997; Menkhurst and Jarman, 2005). Captive breeding programs are underway and reintroductions were recently commenced in Victoria, where the species is nearly extinct. Possible disease risks are an important consideration when implementing expensive captive breeding and translocation programs (Cleaveland et

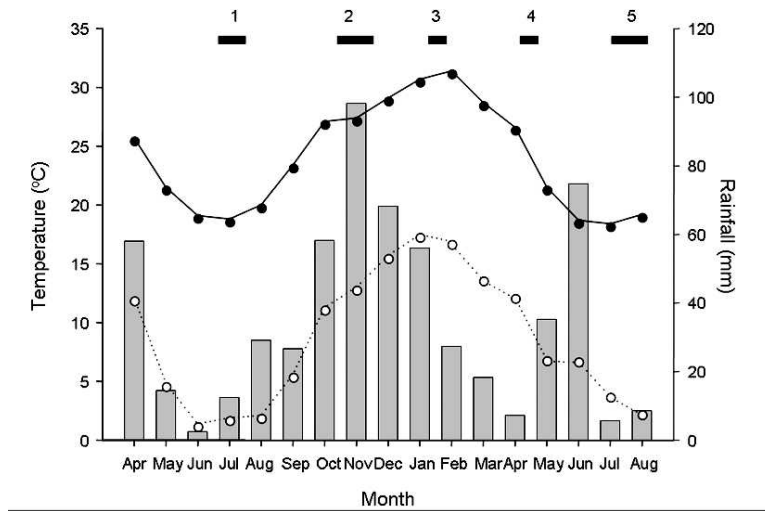


FIGURE 1. Total monthly rainfall (vertical bars) at Tannymorel and mean monthly maximum (●) and minimum (○) temperatures at Warwick from April 2004 to August 2005. Data were supplied by the Bureau of Meteorology, Australian government. Timing and duration of trapping periods are shown with horizontal bars.

al., 2001). It may be possible to prevent declines in introduced populations due to disease by identifying and managing pathogens associated with high mortality and/or reduction of reproductive success in the host.

In this study, we provide a detailed description of parasite burdens in a wild population of the brush-tailed rock-wallaby. We report on the variation in endo- and ectoparasite burdens during a 1 yr study and the seroprevalence of the introduced parasite *T. gondii*. We also investigate the impacts of parasite burdens on the health of individuals and the population.

#### MATERIALS AND METHODS

The Hurdle Creek, Farm Creek, and Farm Creek East brush-tailed rock-wallaby colonies are located on privately owned land 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). The Hurdle Creek colony consists of 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 site has relatively warm wet summers and cool dry winters. Rainfall data and temperature statistics from 3 mo before the start of the study until the end of trapping period 5 are presented in Figure 1. Rainfall throughout the study period was below the long-term average for the district, particularly during the first winter (Bureau of Meteorology, Australian Government).

Animals were caught in baited treadle traps over five trapping periods: 1=July 2004 (Hurdle Creek), 2=November 2004 (Hurdle Creek), 3=January/February 2005 (Hurdle Creek and Farm Creek East), 4=April–May 2005 (Hurdle Creek and Farm Creek East), and 5=July/August 2005 (all colonies). During trapping period 1, body condition, reproductive data, and fecal samples were collected. In subsequent trapping periods, ectoparasite data and blood samples were also collected from each animal. Within each trapping period, each wallaby was only examined on the first occasion that it was trapped.

A microchip (LifeChip/Digivet.com Pty. Ltd., Baulkham Hills, NSW, Australia) was injected subcutaneously into each trapped animal, enabling identification of retrapped animals. 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, and the condition index

was estimated for each animal as its residual from a regression of  $\log_e(\text{weight})$  on  $\log_e(\text{hind foot length})$  using the 154 animal examinations from Hurdle Creek where weight and foot length data were collected, as described in Barnes et al. (2008b).

The coat of each animal was parted five times along the length of the body, and presence or absence of lice on the animal was noted. The whole body surface was examined for ticks, and sites and numbers of ticks were recorded. Representative ectoparasites were removed and stored in 70% ethanol for later identification. Ticks were identified using the descriptions of Roberts (1970), and lice were identified using the descriptions of Clay (1981). The sparsely haired inguinal and axillary regions were each scored from 0 to 3 (0=no visible skin lesions, 1=localized area less than 2 cm in diameter with small papules and no erythema, 2=localized area less than 2 cm in diameter with papules, mild erythema, and/or mild crusting, 3=area greater than 2 cm in diameter with papules and erythema and crusting). A total skin lesion score was then calculated as the sum of the values for the axillary and inguinal regions. Skin scrapings were taken from the inguinal and/or axillary regions of a subsample of animals with and without lesions at these sites. Mites were identified using the descriptions of Domrow (1988). Parasites were deposited in the Parasite Collection, Australian National Wildlife Collection, CSIRO Canaberra (*Ixodes holocyclus*, AR 1551; *Haemaphysalis bancrofti*, AR 1552; *Heterodoxus octoseriatus*, AR 1553; *Breinvia spelaea*, N5456).

Blood samples (2–4 ml) were collected from either the lateral coccygeal vein or jugular vein. The methods and results of hematologic and serum biochemistry analyses are reported in Barnes et al. (2008a). Values for eosinophil count, hemoglobin (Hb), red cell count (RCC), packed cell volume (PCV), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), protein, albumin, and globulin were used in statistical analyses. Blood smears were examined under bright-field microscopy (Olympus BH2) at 400 $\times$  magnification. Microfilariae were counted and recorded as total number per smear. The first serum sample from each animal was screened for antibodies to *Toxoplasma gondii* at 1/40 and 1/4,000 dilution using a modified agglutination test according to the manufacturer's instructions (Toxo-Screen DA, Biomerieux, France); endpoint titers were determined for all positive samples. Serum from an eastern grey kangaroo from which *Toxoplasma* lesions had been identified

postmortem was used as a positive macropodid control. Positive and negative sera supplied by the manufacturer provided further controls.

A fresh fecal sample was collected from the trap of each captured animal and stored at 4 C until examination. The consistency of each fecal sample was noted, and a 2 g fraction was examined using the McMaster method (Colville, 1991) to determine the number of helminth eggs (epg) and coccidian oocysts (opg) per gram of feces. Approximately 5 g of feces from samples with high oocyst counts were placed in 2–2.5% aqueous (w/v) potassium dichromate to enable sporulation and preservation of oocysts as described by Duszynski and Wilber (1997). Oocysts were identified using the descriptions of Barker et al. (1988).

One animal died during the course of the study and was necropsied within 2 hr of death. Filarioid parasites recovered from this animal were stored in 70% ethanol and later identified using the descriptions of MacKerras (1962).

Statistical analyses were performed using Stata version 9 (StataCorp, College Station, Texas, USA). Fecal, ectoparasite, and filarioid parasite data and condition indices were analyzed using generalized estimating equations (GEE) to account for clustering of the outcome variable by animal. Only data from the Hurdle Creek colony were used in analyses because data from Farm Creek were only collected on one occasion, and the sample size from Farm Creek East was very small (13 animal examinations involving eight animals). Before analysis, fecal data were log transformed after adding 1 to each value. Distributions of both tick (all life stages combined) and microfilaria counts were compared with those expected using both Poisson and negative binomial distributions. The Poisson distributions used an estimate of the Poisson mean, and the negative binomial distributions used the same mean and an estimate of the overdispersion parameter.

GEE models were initially run using univariable analyses to assess associations among trapping period, sex, and age class and each outcome variable based on Wald *P*-values. Where two factors were significant at *P*<0.05 on univariable screening, both were fitted simultaneously, and each was retained in the final model if significant. Continuous outcome variables (fecal data and condition indices) were modeled using normally distributed residuals and identity link functions. Binary outcome variables (presence/absence of lice and microfilariae, and total rash scores categorized as less than 3 or equal to or greater

than 3) were modeled using binomially distributed residuals and logit links. Outcome variables that followed a negative binomial distribution were modeled using negative binomially distributed residuals and log links. For these GEE models, the value of  $\alpha$  specified was the estimate from a negative binomial regression model with the same exposure variable(s) with standard errors adjusted for clustering of the outcome variable by animal. Exchangeable correlation structures and Huber/White/sandwich estimates of variance (Rogers, 1993) were used for all GEE models, and time was fitted as a categorical variable. The estimated correlation between any pair of time points within an animal ( $\rho$ ) was recorded for the final model for each of the continuous outcome variables.

Associations between parasitism and selected blood variables and condition indices were also assessed using GEE models, as described already, using normally distributed residuals and identity link functions. Only those associations considered biologically plausible a priori were selected for these analyses. Associations were assessed between fecal egg count and oocyst count as putative explanatory variables and eosinophil count, Hb, RCC, PCV, AST, ALP, GGT, protein, albumin, and globulin as outcome variables. Blood variables were log transformed when required to reduce skewness. Associations were modeled with adjustments for age class, sex, trapping period, and/or method of restraint if these had been previously associated with the outcome variable (Barnes et al., 2008a). Univariable logistic regression was undertaken to investigate the association between the presence of mites in skin scrapings as a putative explanatory variable and the presence of skin lesions as the outcome variable. The associations between oocyst count as a putative explanatory variable and fecal egg count as the outcome variable and the reverse were assessed using GEE models, as described already, with adjustments for trapping period, sex, and age class.

## RESULTS

During the study, 71 individual animals were trapped. Apart from skin lesions, no clinical abnormalities were detected on any animal examinations. Condition data and fecal samples were collected at 179 animal examinations; data from the 154 animal examinations from 51 animals from Hurdle Creek were used for statistical

analyses. Blood samples and data on ectoparasites were collected at 141 of these 179 animal examinations from a total of 64 individual animals; of these, the 115 animal examinations from 44 individual animals at Hurdle Creek were used for statistical analyses. Skin scrapings were taken from 31 animals; the 18 scrapings from animals at Hurdle Creek were used for statistical analyses.

Three of the 64 (4.7%) animals tested had positive titers to *T. gondii*. Two were adult females from Hurdle Creek (titers 120, 360), and the other was an adult male from Farm Creek (titer 360).

Condition indices were analyzed from 154 animal examinations and varied from  $-0.243$  to  $0.205$  (mean  $\pm$  standard deviation [S.D.]  $0.002 \pm 0.083$ ). Condition estimates were highly correlated within individual animals between any two trapping periods ( $\rho=0.55$ ). Overall, there was a significant effect of trapping period ( $P=0.016$ ) on condition index, and higher mean condition indices were found in trapping periods 2 and 4. However, point estimates for each trapping period were imprecise, and therefore the magnitude of these effects could not be described precisely (Table 1).

*Eimeria* oocysts were found in 95.0% of fecal samples (170/179). Both *Eimeria sharmani* and *Eimeria petrogale* were identified. A further small coccidian present in some samples was not identified. Fecal oocyst counts were highly variable (range: 0–39,429 opg, mean  $\pm$  S.D.:  $6,121 \pm 7,850$  opg) and varied between trapping periods ( $P<0.001$ ). Counts within individual animals were weakly correlated between any two trapping periods ( $\rho=0.14$ ). Counts were lowest in trapping period 1 and highest in trapping periods 2 and 3. Oocyst counts were not significantly associated ( $P>0.190$ ) with sex or age class (Table 1). However, the counts for subadults were estimated to be 1.62 times higher than that for adults (95% confidence interval [CI] 0.79, 3.32).

Strongylid eggs were detected in most

TABLE 1. Associations among selected risk factors and condition index, fecal egg count, and oocyst count using data from brush-tailed rock-wallabies trapped over five trapping periods from July 2004 to August 2005 at Hurdle Creek, southeast Queensland.

Outcome variable <i>Exposure variable</i>	No. of animals	Arithmetic mean	Ratio of geometric means (95% CI)	P-value
Condition index				
<i>Trapping period</i>				0.016
1	32	-0.062	1.00	
2	27	0.0297	1.03 (0.99, 1.06)	
3	28	-0.0084	0.98 (0.96, 1.01)	
4	34	0.0183	1.01 (0.98, 1.05)	
5	33	-0.0196	0.98 (0.95, 1.01)	
Fecal egg count (epg)				
<i>Trapping period</i>				<0.001
1	32	991	1.00	
2	27	460	0.50 (0.38, 0.67) <sup>a</sup>	
3	28	661	0.54 (0.30, 0.96) <sup>a</sup>	
4	34	578	0.71 (0.52, 0.97) <sup>a</sup>	
5	33	526	0.51 (0.33, 0.80) <sup>a</sup>	
<i>Age</i>				0.010
Adult	93	740	1.00	
Subadult	61	507	0.58 (0.39, 0.88) <sup>b</sup>	
<i>Sex</i>				0.980
Female	93	682	1.00	
Male	61	595	0.99 (0.67, 1.51)	
Fecal oocyst count (opg)				
<i>Trapping period</i>				<0.001
1	32	748	1.00	
2	27	8,006	13.25 (4.62, 37.96)	
3	28	10,040	26.47 (11.74, 59.69)	
4	34	3,734	5.24 (2.06, 13.31)	
5	33	3,645	5.35 (2.28, 12.58)	
<i>Age</i>				0.191
Adult	93	4,550	1.00	
Subadult	61	5,660	1.62 (0.79, 3.32)	
<i>Sex</i>				0.712
Female	93	4,916	1.00	
Male	61	5,102	0.87 (0.43, 1.79)	

<sup>a</sup> Adjusted for age.

<sup>b</sup> Adjusted for trapping period.

fecal samples (98.9%, 177/179). Although several different morphotypes were seen, identification to genus level was not possible. The characteristic egg of *Globocephalolides trifidospicularis* (Beveridge et al., 1985) was not identified in any sample. Fecal egg counts were highly variable (range: 0–2,362 epg, mean  $\pm$  S.D.:  $568 \pm 432$  epg) and varied between trapping periods ( $P < 0.001$ ) and age classes of animals ( $P = 0.010$ ), and counts were highest in trapping period 1 and in

samples from adult animals (Table 1). Fecal egg counts were weakly correlated within individual animals between any two trapping periods ( $\rho = 0.17$ ).

Only one fecal sample was abnormally soft, with the pellets surrounded by mucus; the fecal egg count for this animal at this examination was 313 epg, and the oocyst count was 1875 opg.

One male and one female adult *Breintlia spelaea* were found in the mesentery of the dead animal at postmortem examina-

TABLE 2. Associations between selected putative risk factors and the presence of microfilaria or lice and total skin lesion score of  $\geq 3$  using data from brush-tailed rock-wallabies trapped from November 2004 to August 2005, at Hurdle Creek, southeast Queensland.

Outcome variable <i>Exposure variable</i>	No. examined	% infected	Odds ratio (95% CI)	P-value
<b>Microfilaria</b>				
<i>Trapping period</i>				0.003
2	21	61.9	1.00	
3	28	25.0	0.19 (0.05, 0.69) <sup>a</sup>	
4	33	24.2	0.19 (0.05, 0.64) <sup>a</sup>	
5	33	24.2	0.19 (0.05, 0.73) <sup>a</sup>	
<i>Age</i>				<0.001
Adult	67	46.3	1.00	
Subadult	48	10.4	0.14 (0.04, 0.50) <sup>b</sup>	
<i>Sex</i>				0.943
Female	64	31.3	1.00	
Male	51	31.4	0.71 (0.39, 2.39)	
<b>Lice</b>				
<i>Trapping period</i>				0.005
2	21	66.7	1.00	
3	27	22.2	0.14 (0.04, 0.50)	
4	34	41.1	0.34 (0.10, 1.17)	
5	33	27.3	0.18 (0.06, 0.61)	
<i>Age</i>				0.987
Adult	67	37.3	1.00	
Subadult	48	37.5	1.01 (0.46, 2.22)	
<i>Sex</i>				0.239
Female	64	42.2	1.00	
Male	51	31.4	1.59 (0.29, 1.36)	
<b>High total skin lesion score<sup>c</sup></b>				
<i>Trapping period</i>				<0.001
2	21	61.9	1.00	
3	26	46.2	0.56 (0.18, 1.70)	
4	34	0.0	0.00 (0.00, 0.00)	
5	33	3.0	0.02 (0.00, 0.16)	
<i>Age</i>				0.340
Adult	66	25.8	1.00	
Subadult	48	18.8	0.67 (0.30, 1.51)	
<i>Sex</i>				0.657
Female	65	24.6	1.00	
Male	49	20.4	0.82 (0.35, 1.95)	

<sup>a</sup> Adjusted for age.

<sup>b</sup> Adjusted for trapping period.

<sup>c</sup> Total skin lesion score of  $\geq 3$  relative to  $< 3$ .

tion. No lesions were seen in the surrounding mesentery. Microfilariae, consistent with those described for *B. spelaea*, were found in 31.3% of smears (36/115), and over half of the animals sampled (32/63) had at least one positive smear. Odds of a tested animal having a positive smear were approximately five times higher in trapping period 2 than at any other time

( $P=0.003$ ) and over seven times higher for adults compared to subadults (Table 2;  $P<0.001$ ). Mean counts of microfilariae were greater in trapping period 2 ( $P<0.001$ ) and in adult animals relative to subadults (Table 3;  $P=0.001$ ). Microfilaria counts were overdispersed, approximating a negative binomial distribution ( $\alpha=7.2$ , mean 1.8).

TABLE 3. Associations between selected risk factors and counts of microfilariae and ticks using data from brush-tailed rock-wallabies trapped from November 2004 to August 2005, at Hurdle Creek, southeast Queensland.

Outcome variable <i>Exposure variable</i>	No. of animals examined	Mean count	Ratio of mean counts (95% CI)	<i>P</i> -value
<b>Microfilaria</b>				
<i>Trapping period</i>				
2	21	7.4	1.00	<0.001
3	28	0.6	0.13 (0.04, 0.44) <sup>a</sup>	
4	33	0.4	0.08 (0.03, 0.23) <sup>a</sup>	
5	33	0.5	0.09 (0.03, 0.26) <sup>a</sup>	
<i>Age</i>				
Adult	67	2.9	1.00	0.003
Subadult	48	0.2	0.18 (0.06, 0.56) <sup>b</sup>	
<i>Sex</i>				
Female	64	1.5	1.00	0.682
Male	51	2.0	1.32 (0.35, 4.91)	
<b>Ticks</b>				
<i>Trapping period</i>				
2	19	5.7	1.00	0.018
3	26	5.6	0.97 (0.48, 1.96)	
4	34	1.3	0.23 (0.09, 0.59)	
5	33	4.3	0.76 (0.48, 1.20)	
<i>Age</i>				
Adult	65	3.9	1.00	0.836
Subadult	47	4.0	0.95 (0.59, 1.53)	
<i>Sex</i>				
Female	63	3.9	1.00	0.939
Male	49	4.0	1.01 (0.64, 1.63)	

<sup>a</sup> Adjusted for age.

<sup>b</sup> Adjusted for trapping period.

One species of louse, *Heterodoxus octoseriatus*, was found on brush-tailed rock-wallabies at 37% of animal examinations (43/115). Odds of animals having lice were between three and seven times higher in trapping period 2 relative to other trapping periods ( $P=0.005$ ; Table 2). No animals were heavily infected; only one examination showed more than five lice seen in five coat partings.

Two species of tick, *Ixodes holocyclus* and *Haemaphysalis bancrofti*, were found on animals during 75.8% (88/116) of total animal examinations. Most of the ticks sampled were adults or nymphs, but larvae of both species were identified occasionally, particularly during trapping period 4. Tick counts were overdispersed among animals, approximating a negative binomial distribution ( $\alpha=1.4$ , mean 3.9). Tick counts varied significantly with trapping

period ( $P=0.018$ ) and were lowest in trapping period 4, but they did not vary significantly with sex ( $P=0.939$ ) or age class ( $P=0.836$ ; Table 3).

Ticks were found most frequently on the ears, neck, and eyelids (24.8% of all 115 animal examinations, respectively). Ticks were also recovered, in decreasing order of frequency, from the inguinal area, axillary area, shoulders, thighs, chest, pouch, base of tail, flank, genitalia, nose, mandible, and elbow. Engorged female *I. holocyclus* were associated with granulomas, up to 2 cm in diameter, and these lesions were seen most frequently on the ventral part of the neck. No animals exhibited signs of tick paralysis. A mild localized dermatitis at the point of attachment of other ticks was occasionally seen.

Inflammatory skin lesion scores ranged from 0 to 6 (median 2,  $n=114$ ), and odds

of a high total skin lesion score ( $\geq 3$ ) were greatest in trapping period 2 and lowest in trapping period 4 (Table 2;  $P < 0.001$ ). Skin scrapings were taken from either axillary or inguinal areas of 31 animals (22 with lesions in these areas and nine without lesions). A new species of the mite genus *Thadeua* (M. Shaw, personal comm.) was recovered from 11 animals. Two females of this species are registered in the collection of the Queensland Museum (QMS 83704-5). The new species shares several characters with *Thadeua serrata* Domrow, and, aside from lacking spinose posteroventral setae, it keys to *T. serrata* in Domrow (1988). However, this species is unique in having a pair of large sclerotized patches on the posterolateral corners of the venter. This new species was detected in 41% (9/22) of animals with skin lesions. However, it was also found in 22% (2/9) of animals with no lesions. At Hurdle Creek, mites were detected in 73% (8/11) of animals with skin lesions, and presence of mites was associated with skin lesions in the animals examined from this colony (OR 6.67; 95% CI 0.81, 54.96;  $P = 0.078$ ). However, at Farm Creek, only animals with lesions were scraped, and mites were only seen in 9% (1/11) of these animals. Samples were only taken from two animals at Farm Creek East, neither of which had mites or skin lesions. Larvae of the trombiculid *Guntheria (Derrickiella) queenslandica* were also seen in several scrapings.

Neither fecal egg count nor oocyst count was associated ( $P > 0.099$ ) with any of the following blood variables: Hb, RCC, PCV, AST, ALP, GGT, protein, albumin, or globulin. Observed point estimates for these associations were small and precise; estimated changes in variable per 100 epg and per 1,000 opg and limits of confidence interval were all less than 1% of crude mean. This indicates that if any of these variables are actually associated, such associations are weak. Neither fecal egg counts nor oocyst counts were associated with eosinophil counts. However, the

point estimate for the association between fecal egg count and eosinophil count was less precise, allowing for the possibility that an important association actually exists. Estimates of association between fecal egg count and oocyst count (as explanatory variables) and condition index were all very imprecise, so actual effects are uncertain.

There was a negative association between fecal egg count and fecal oocyst count, such that, for every extra 100 epg, geometric mean opg was lower by a factor of 0.94 (95% CI 0.89, 0.99;  $P = 0.033$ ). Similarly, fecal egg counts were lower with higher oocyst counts.

## DISCUSSION

This study provides data on parasite burdens in a wild population of the brush-tailed rock wallaby. This macropodid hosts a diverse range of parasites, and these parasite burdens are highly variable between animals and within animals over time. At the time of the study, the host population was likely to have been under some stress due to a prolonged drought, but there was little evidence that parasites that had evolved with the host had a significant impact on the wallaby population. Of the two introduced generalist parasites known to cause disease in macropodids, only *E. granulosus* appeared to have a significant impact in this population (Barnes et al., 2008b). The prevalence of *T. gondii* in the study population was low, and antibody positive animals showed no evidence of disease.

This is the first published prevalence estimate of *B. speleae* in the brush-tailed rock-wallaby. Detection of microfilariae in 50% of animals in this study indicates that infection is common in this area. The longevity of adult worms has not been described, but the higher percentage of positive blood smears from older animals suggests that the parasite is long-lived and infection is cumulative or, less likely, that adults have higher new infection rates.



The intermediate host is unknown, but other species of *Breinvia* in Australia and Southeast Asia are transmitted by mosquitoes (Ramachandran and Dunn, 1968; Ho et al., 1973; Yen, 1983). There were no lesions associated with adult worms in the necropsied animal, consistent with other reports of *Breinvia* infections in macropodids (Beveridge, 1976), suggesting that they may not be detrimental to the host.

The high prevalences of coccidia (95.0%) and the *Eimeria* species identified concur with a previous report (Barker et al., 1988). Fecal oocyst counts did not differ significantly between younger and older animals, but the observed mean count was higher for subadult animals. Such a pattern would be consistent with that generally seen in domestic species, where immunity is attained early in life, and oocyst counts are much lower in adult animals (Jolley and Bardsley, 2006). Clinical signs of coccidiosis were not seen in any of the brush-tailed rock-wallabies, despite some very high oocyst counts seen in periods of warm weather following significant rain (trapping periods 2 and 3; Fig 1). There was also no association between counts and blood variables such as hemoglobin or protein levels.

Higher fecal egg counts were seen in adult compared to subadult animals, suggesting that there is no important age-related or acquired immunity to adult worms. The significant difference between strongylid egg counts from trapping periods 1 and 5, from the same months in successive years, highlights the complexity of the epidemiology of nematode infections in macropodids. There was no evidence to suggest that the nematode burdens found in this study were pathogenic to brush-tailed rock-wallabies under these conditions.

Ticks and lice were commonly found on the brush-tailed rock-wallabies, and there was some variation in prevalence and intensity of infection between the trapping periods. However, burdens were low compared to those reportedly associated

with illthrift (either as cause or effect) in other species (Gemmell et al., 1991; Turni and Smales, 2001), and, with the exception of dermatitis at the point of attachment of engorged adult female *I. holocyclus*, clinical signs were not evident. No clinical signs of tick paralysis were seen in animals infected with engorged *I. holocyclus*. It is possible that these macropodids develop immunity to the toxin as result of continual exposure, as suggested by Seddon and Albiston (1968) for bandicoots. These findings suggest that the burdens of lice and ticks reported in this study have minimal impact on overall health. A possible loss or failure to establish immunity to the toxin produced by *I. holocyclus* is, however, an important consideration for captive breeding or translocation programs if animals are to be released in areas where this tick is endemic.

Similar skin lesions to those seen in the brush-tailed rock-wallabies have been recorded from the axillary and inguinal regions of the Proserpine rock-wallaby (*Petrogale persephone*) and attributed to *Thadeua serrata* (Begg et al., 1995; Skerratt et al., 2007), a mite from the same genus as the new species identified from these rock-wallabies. Although lesions in the brush-tailed rock-wallabies were associated with the presence of mites, some animals without lesions also had mite-positive skin scrapings, suggesting that these lesions have multifactorial origins.

Imprecise estimates of association between both fecal egg count and oocyst count (as explanatory variables) and condition index preclude conclusions about the effects of either of these infections on the condition of animals. However the fluctuation of parasite counts within individuals suggests that, in the study population, any adverse effects of these parasite burdens would not always be prolonged. This may explain the absence of associations with condition index in this study. However, when assessing these and other associations between parasite burdens and

health of individuals, the study design may have resulted in selection bias if only relatively healthy animals that were actively foraging were trapped, whereas those that were unhealthy or had died were not trapped. These problems were discussed by McCallum (1994) in relation to the quantification of the pathogenicity and impact of parasites on host populations; he suggested that experimental manipulation of parasite burdens is required to provide definitive answers. This selection bias would also be reduced with more frequent trappings of entire populations. Clearly, these approaches were beyond the scope of this study, but the data gathered from apparently healthy animals in this population provide a baseline that will be extremely useful for monitoring the health of other populations of brush-tailed rock-wallabies when conservation management plans, such as the recent reintroduction of the species to the Grampians, are put into practice.

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