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Effects of Habitat, Host Sex and Age on the Parasites of *Trichosurus caninus* (Marsupialia : Phalangeridae) in North-Eastern New South Wales

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Abstract

The condition of 57 *Trichosurus caninus* (Ogilby), and their ectoparasites, endoparasites and associated pathology were examined; ages and habitat status of these animals were known. Condition scores for females were greater ($P < 0{\cdot}005$) than those for males. Seven ectoparasite species (two ticks, five mites), two protozoan and seven helminth (one cestode, six nematodes) species were identified in *T. caninus* from both preferred and peripheral habitats. Prevalence of *Amplicaecum robertsi* (Sprent & Mines) larvae and the oxyurid nematode *Adelonema trichosuri* (Johnston & Mawson) were greater ($P < 0{\cdot}05$) in *T. caninus* from peripheral habitat than in preferred-habitat animals. Greater burdens ($P < 0{\cdot}05$) of the tick *Ixodes holocyclus* Neumann, two mites *Trichosurolaelaps dixoa* Domrow and *T. crassipes* Womersley, and the trichostrongylid nematode *Paraustrostrongylus trichosuri* Mawson were found in peripheral-habitat *T. caninus*. Subadult males harboured greater *Paraustrostrongylus* burdens ($P < 0{\cdot}05$) than did subadult females or adult animals. Three *Trichosurus vulpecula* (Kerr) sympatric with peripheral-habitat *T. caninus* were also examined. One tick *I. trichosuri* Roberts, one mite *T. crassipes* and four helminth species: *Bertiella trichosuri* Khalil, *A. robertsi*, *Paraustrostrongylus trichosuri* and *Parastrongyloides trichosuri* Mackerras, were identified. The cestode *B. trichosuri* was recovered from the three *T. vulpecula* and four peripheral-habitat *T. caninus*, but only from one *T. caninus* from preferred habitat.

Larval *A. robertsi* caused focal eosinophilic cholangiohepatitis with dilatation of affected bile ducts in livers of both *Trichosurus* spp. Eosinophilic vasculitis of hepatic portal veins was associated with ?*Sprattia venacavincola* (Spratt & Varughese) and focal granulomatous splenitis with sequestered microfilariae in *T. caninus*. Also, ?*Marsupostrongylus minesi* Spratt in dilated alveoli caused mild pulmonary inflammation. There were no pathological changes associated with intestinal tract parasites.

Free corticosteroid levels in preferred-habitat female *T. caninus* were greater ($P < 0{\cdot}05$) than in those from peripheral habitat. This correlated with hyperplastic changes in adrenal glands of these females, but not with parasite burdens.

Introduction

Since 1967 natural populations of two phalangers, the mountain possum *Trichosurus caninus* (Ogilby) and brush-tailed possum *T. vulpecula* (Kerr) have been studied (How 1976, 1981). Population strategies for these species differ markedly (How 1978); *T. caninus* is the more stable species (*K*-selected) and *T. vulpecula* the more adaptable (*r*-selected) (Pianka 1970). Interspecific differences and sexual dimorphisms in blood parameters occur and seasonal responses differ (Barnett *et al.* 1979*a*). Preferred and peripheral habitat types were known; in peripheral habitat *T. caninus* has blood parameters and seasonal changes that resembled those

of *T. vulpecula*; a physiological shift towards *r*-selection in these *T. caninus* was suggested (Barnett *et al.* 1979*b*). Between-habitat differences in organ weights and numbers of corpora lutea in *T. caninus* at postmortem examination provided additional evidence that these subpopulations were distinct (Barnett *et al.* 1982).

In this communication, the parasites and associated pathology in 57 *T. caninus* are presented; effects of habitat, and of sex and age of host on the prevalence and intensity of these infections are examined. The parasites of three sympatric *T. vulpecula* are included. Since parasite burdens may reflect the effects of environmental or social stressors on host populations (Esch *et al.* 1975), parasite burdens in *T. caninus* could provide additional parameters by which these subpopulations may be differentiated. Host susceptibility to parasites is often mediated through the immune system, and changes in its status may be detected by blood corticosteroid analysis (Esch *et al.* 1975; Stein *et al.* 1976). Therefore, plasma corticosteroid levels were also determined.

Materials and Methods

Study Area

The study area, of 118 km^2, is at Clouds Creek in north-eastern New South Wales (Barnett *et al.* 1979*a*). Preferred habitat for *T. caninus* was closed and tall open forest, and for *T. vulpecula* it was grazed woodland and open forest (Barnett *et al.* 1976, 1979*b*). Both species were sympatric in adjacent pine plantations or open forest in their peripheral habitats (Barnett *et al.* 1979*b*).

Animals

Animals were trapped in May–June 1977 and blood samples were collected at the trap site (Barnett *et al.* 1979*a*). They were transported to a forestry building and killed by exsanguination under ether anaesthesia following an intraperitoneal injection with sodium pentabarbitone. Blood was collected and various organs removed and weighed (Barnett *et al.* 1982).

A total of 57 *T. caninus*, 29 females and 28 males, was examined. Of these, 34 were known-age animals marked as young (Barnett *et al.* 1982) and the others were aged by cementum annuli (Pekelharing 1970; Barnett *et al.* 1982). *T. caninus* aged < 3 y were classified as subadults and those > 3 y as adults. Previous trap records since 1968 were used to assign each *T. caninus* to either preferred (34) or peripheral (23) habitat as discussed previously (Barnett *et al.* 1979*b*, 1982).

Three male *T. vulpecula* of 2, 7 and 9 y old were also examined. Each was trapped in habitat sympatric with peripheral-habitat *T. caninus*.

Postmortem Examination

The external condition, including the presence and severity of 'rump wear', was noted for each animal (rump wear is a condition characterized by short grey fur, often with focal patches of darker regrown hair, in the lower back region of *T. caninus*). Fat stores in peritoneal and thoracic depot areas were used to assess condition as poor, fair, good, very good or excellent and scored from 1 to 5, respectively. Representative samples from most organs and any gross lesions were fixed in 10% formol saline for histological examination. Trimmed tissues were paraffin-embedded, sectioned at 6 μm and stained with haematoxylin and eosin.

Parasitological Examination

Ectoparasites on the pelage were removed and fixed in 70% ethanol. After postmortem examination, each carcass was placed in a plastic bag and allowed to cool overnight. The next day the fur was combed vigorously for several minutes over white paper to recover ectoparasites. Each carcass was then skinned. Later, a portion of the central back fur, 8 by 4 cm, from 10 *T. caninus*, was digested in 10% potassium hydroxide to recover mites.

The peritoneal cavity was examined for filarioid nematodes and the liver for ascaridoid larvae. Hepatic nodules were dissected and the nematodes counted and fixed in hot 70% ethanol. The proximal

duodenum (first 20 cm) was opened and its mucosa scraped into a white tray containing water. Nematodes were counted as they were drawn up in a pasteur pipette and transferred to 70% ethanol for subsequent identification. The distal small intestine was flushed with water to recover any cestodes; after relaxation in cold water for 12 h, these were fixed in 5% formol saline. The distal 10 cm of the caecum was opened and examined grossly for pinworms. These nematodes were not counted and only a relative assessment (light, moderate, heavy) of their burden was made. Faecal samples from seven *T. caninus* were stored in 2·5% potassium dichromate and later examined for coccidian oocysts.

Four 4-cm segments of the proximal duodenum of two *T. caninus* were clamped, and Bouin's fluid injected into their lumina, to assess the pathological effects of intestinal nematodes. After 24 h in Bouin's solution, these segments were transferred to 70% ethanol. Later the trimmed tissue with attached nematodes was double-embedded in celloidin, then paraffin. Sections were then cut and stained as described above.

Representative specimens of helminth species recovered from *T. caninus* have been deposited in the Australian Helminth Collection, formerly the University of Adelaide, Department of Zoology Helminth Collection, currently housed in the South Australian Museum, Adelaide.

Corticosteroid Analyses

Plasma total corticosteroid (TC) concentrations were determined on samples taken at the site of capture by the competitive protein-binding assay of Murphy (1967). The maximum corticosteroid-binding capacity (MCBC), as a measure of transcortin concentration, was determined by the method of Doe *et al.* (1964) with charcoal separation of the free and bound hormone (Pegg and Keane 1969). Assuming a high-affinity binding constant of $4{\cdot}03 \times 10^7$ 1 mol^{-1} at 36°C and an albumin bound : free ratio of 0·785 as in *T. vulpecula* (Khin Aye Than and McDonald 1976), the concentration of plasma free corticosteroid (FC) was calculated (Tait and Burstein 1964).

Statistical Analyses

Analysis of variance on log-transformed data [$\ln (x+1)$] was used to establish significant differences in parasite burdens between animals in various habitat, sex and age classes. Where age had no significant effect, the data were combined and analysed for differences between sex and habitats. Where prevalence differed between habitats, χ^2 contingency tables with one degree of freedom were used to determine significance. Spearman's rank correlation coefficient adjusted for tied values was used to assess age effects when sex and age interacted. If a significant interaction was found, the *a posteriori* multiple comparison test with experimental-wise error rate was used to determine which parameter was involved (Daniel 1978).

Results

Condition of Animals

Rump wear was noted in 23 (40%) *T. caninus* and no *T. vulpecula*. This condition was more common in males (54%) than in females (28%), but this difference was not significant ($P > 0{\cdot}05$). Tick bite lesions, a common finding in the inguinal region of *T. caninus*, were also recorded on one *T. vulpecula*.

Condition scores for female *T. caninus* (4·6 in preferred and 4·0 in peripheral habitats) were greater ($P < 0{\cdot}005$) than those for males (3·2 in preferred and 2·8 in peripheral habitats). The cortices of most *T. caninus* kidneys (72%) were pale to dark yellow in color; none of the *T. vulpecula* kidneys was affected. This pigment leached out in formol saline or 70% ethanol. Significant effects of age, sex or habitat on its prevalence were not found, but pigments in kidneys of preferred-habitat females were correlated ($P < 0{\cdot}02$) with the occurrence of pigment granules in the hepatocytes of the liver. This pigment accumulated with age ($P < 0{\cdot}02$) in *T. caninus*, particularly among males in both preferred and peripheral habitats ($P < 0{\cdot}05$). Differences in the occurrence of hepatic pigment between sexes or

Table 1. Prevalence and mean burdens of ectoparasites and endoparasites recovered from *T. caninus* in its preferred and peripheral habitat at Clouds Creek, N.S.W.

A, preferred habitat. B, peripheral habitat. * New host record. ND, not determined. Data for *T. crassipes* and *T. dioxa* are combined

Parasite	Site in host or recovery	No. affected A	No. affected B	No. examined A	No. examined B	Prevalence (%) A	Prevalence (%) B	Parasite burden Mean	Parasite burden Range
Acari									
Ixodidae									
Ixodes holocyclus Neumann, 1899	Pelage	32	22	34	22	94	100	8	1–39
Ixodes tasmani Neumann, 1899	Pelage	3	3	34	22	9	14	1	1–2
Dermanyssidae									
Trichosurolaelaps crassipes Womersley, 1956	Pelage	25	18	34	22	74	82	7	1–37
Trichosurolaelaps dixoa Domrow, 1972	Pelage								
Haemolaelaps penelope Domrow, 1964	Skin digest	(1)		6	4	(10)		ND	—
Atopomelidae									
**Atellana papilio* Domrow, 1958	Skin digest	(1)		6	4	(10)		ND	—
**Petrogalochirus dycei* (Domrow, 1960)	Skin digest	(1)		6	4	(10)		ND	—
Protozoa									
Eimeriidae									
**Eimeria* sp.	Faeces	1	2	2	5	—		—	—
**Sarcocystis* sp.	Tongue	1	1	29	15	3	7	—	—
Cestoda									
Anoplocephalidae									
Bertiella trichosuri Khalil, 1970	Small intestine	1	4	34	23	3	17	1	1–2
Nematoda									
Anisakidae									
Amplicaecum robertsi Sprent & Mines, 1960	Bile ducts	14	16	34	23	41	70	13	1–216
Trichostrongylidae									
**Paraustrostrongylus trichosuri* Mawson, 1973	Small intestine	34	23	34	23	100	100	232	8–1831
Strongyloididae									
**Parastrongyloides trichosuri* Mackerras, 1959	Faeces	34	23	34	23	100	100	ND	—
Oxyuridae									
**Adelonema trichosuri* (Johnston & Mawson, 1938)	Caecum	7	12	34	23	21	52	ND	—
Angiostrongylidae									
?*Marsupostrongylus minesi* Spratt, 1978	Lung	2	3	2	3	—		ND	—
Onchocercidae									
?*Sprattia venacavincola* (Spratt & Varughese, 1975)	Hepatic portal vein	1	0	34	23	3	0	ND	—

habitats were not significant when analysed separately. Lymphoid nodules surrounding sublobular veins in the hepatic parenchyma of both *Trichosurus* spp. were a common feature and were considered normal.

Parasites and Associated Pathology

The prevalence of parasitic infections in preferred- and peripheral-habitat *T. caninus*, and mean burdens of parasites recovered, are presented in Table 1; those parasites identified in the three *T. vulpecula* examined are given in Table 2.

Table 2. Ectoparasites and endoparasites recovered from *T. vulpecula* in an area sympatic with peripheral-habitat *T. caninus* at Clouds Creek, N.S.W.

*New host record. ND, not determined. Site in host: P, pelage; SI, small intestine; BD, bile ducts

Parasite	Site in host	Number affected	Number examined	Parasite burden Mean	Parasite burden Range
Acari					
Ixodidae					
Ixodes trichosuri Roberts, 1960	P	3	3	7	1–17
Dermanyssidae					
Trichosurolaelaps crassipes Womersley, 1956	P	3	3	12	1–33
Cestoda					
Anoplocephalidae					
Bertiella trichosuri Khalil, 1970	SI	3	3	2	1–4
Nematoda					
Anisakidae					
**Amplicaecum robertsi* Sprent & Mines, 1960	BD	3	3	ND	–
Trichostrongylidae					
Paraustrostrongylus trichosuri Mawson, 1973	SI	3	3	20	14–29
Strongyloididae					
Parastrongyloides trichosuri Mackerras, 1959	SI	3	3	ND	–

(i) *Ticks*

Of 457 *Ixodes* spp. recovered, only five were adults, and the majority were either nymphs (223) or larvae (229). Peripheral-habitat *T. caninus* had greater tick burdens ($P < 0{\cdot}01$) than did those from preferred habitat (Table 3); there were no significant sex or age effects. Ticks or lesions were commonly found on the scrotum of males, in the pouch of females, and occasionally beneath the mandible or ears. Focal dermatitis with mild oedema at tick attachment sites and older scars with scab formation were found, but there was no correlation between the numbers of ticks recovered and the observed scars.

One male *T. vulpecula* had subcutaneous oedema of the right forelimb extending to the footpad. On postmortem examination, oedema and focal tenosynovitis were found in association with an embedded female *I. trichosuri*.

(ii) *Mites*

In addition to light burdens of *Trichosurolaelaps dixoa* on *T. caninus*, four species were identified in digested skin samples (Table 1). Mean burdens of

Trichosurolaelaps spp. on peripheral-habitat males were greater ($P < 0{\cdot}01$) than those on other males or females (Table 3). There was no age effect ($P > 0{\cdot}05$), and when age was excluded, between-habitat differences (peripheral > preferred) were significant ($P < 0{\cdot}01$). No association was found between the presence or severity of rump wear and the number of mites recovered.

(iii) *Protozoa*

Sporulated oocysts detected in faeces of *T. caninus* measured $39{\cdot}1 \pm 0{\cdot}4 \times 19{\cdot}7 \pm 0{\cdot}2$ μm ($n = 42$) and contained four sporocysts, $15{\cdot}4 \pm 0{\cdot}4 \times 10{\cdot}0 \pm 0{\cdot}1$ μm ($n = 7$): the micropyle was $3{\cdot}8$ μm ($n = 5$) in size. Sarcocysts in sections of tongue from *T. caninus* measured up to 220×70 μm in size, and no host reaction was associated with them.

Table 3. Effects of sex and habitat on tick (*Ixodes* spp.) and mite (*Trichosurolaelaps* spp.) burdens recovered from *T. caninus*

Values are mean numbers of parasites per individual, ± standard errors; sample sizes are given in parentheses. Superscript letters indicate pairs of values in the same column that differed significantly ($P < 0{\cdot}01$) by analysis of log-transformed data

Sex	Habitat	Ticks	Mites
Females	Preferred	$3{\cdot}8 \pm 0{\cdot}8$ (*17*)	$2{\cdot}9 \pm 0{\cdot}7$ (*17*)
	Peripheral	$11{\cdot}0 \pm 2{\cdot}7$ (*12*)	$6{\cdot}3 \pm 1{\cdot}7$ (*12*)
Males	Preferred	$5{\cdot}8 \pm 1{\cdot}7$ (*17*)	$4{\cdot}4 \pm 1{\cdot}2$ (*17*)
	Peripheral	$13{\cdot}8 \pm 3{\cdot}6$ (*10*)	$11{\cdot}8 \pm 3{\cdot}5$ (*10*)
Combined	Preferred	$^{A}\ 4{\cdot}8 \pm 0{\cdot}9$ (*34*)	$^{A}\ 3{\cdot}6 \pm 0{\cdot}7$ (*34*)
Combined	Peripheral	$^{A}12{\cdot}3 \pm 2{\cdot}2$ (*22*)	$^{A}\ 8{\cdot}8 \pm 1{\cdot}9$ (*22*)

(iv) *Cestoda*

Bertiella trichosuri was more prevalent in *T. caninus* from peripheral habitat (Table 1) and was recovered from the *T. vulpecula* examined in the same area (Table 2). Burdens were light in both hosts and no lesions were attributed to this infection.

(v) *Nematoda*

Pale nodules 0·5–2·0 cm in diameter were commonly found on the parietal surface of the liver. On dissection, these were dilated bile ducts that contained coiled third-stage larvae of *Amplicaecum robertsi* up to 9 cm long, often singly but also in pairs (Fig. 1). In peripheral-habitat *T. caninus* the prevalence of *A. robertsi* was greater ($P < 0{\cdot}05$) than in animals from preferred habitat (Table 1); prevalence in females and males was similar ($P > 0{\cdot}05$). Most *T. caninus* harboured from one to four worms (mean, $6{\cdot}8 \pm 4{\cdot}0$) but burdens of 17, 82 and 216 were recovered from three females. In the animal with 216 worms the parasites weighed 28 g, approximately one-third that of the enlarged liver. Yet this female was in good condition and rearing a pouch young.

Lesions associated with *Amplicaecum* infection were focal, and most of the hepatic parenchyma was normal. Focal hepatocyte necrosis and haemorrhage with eosinophil infiltration associated with migrating larvae (Fig. 2) were found in only six *T. caninus* and two *T. vulpecula*. Larvae migrated only 1–2 cm, so tracks were not extensive. Eosinophil infiltration and haemosiderin-laden macrophages were commonly seen in adjacent portal tracts. In healed migratory tracks there was a multinucleate giant cell reaction to remaining debris, as well as eosinophil and mononuclear cell infiltrations with early fibroplasia surrounding the lesion (Fig. 3). Focal granulomatous inflammation was noted in four *T. caninus* and two *T. vulpecula*. Focal eosinophilic cholangitis with periductal fibrosis and luminal dilatation were associated with larvae in the bile ducts. Lesions were found in 31 (54%) *T. caninus* and three *T. vulpecula*. The bile duct mucosa was usually thin and often incomplete; regeneration to re-establish an intact epithelium was frequently seen. Mechanical dilatation of bile ducts in response to growing larvae and possible toxic excretory products caused mucosal necrosis and haemorrhage (Fig. 4). Haemosiderin was seen in thickened fibrous duct walls of 15 (26%) *T. caninus* but in none of the *T. vulpecula* examined.

Table 4. Effects of sex, age and habitat on *Paraustrostrongylus trichosuri* burdens in *T. caninus* at Clouds Creek, N.S.W.

Values are mean numbers of parasites per individual, ± standard errors; sample sizes are in parentheses. Superscript letters: [A]pairs of values in the same column that differed significantly ($P < 0{\cdot}05$) by analysis of log-transformed data; [B]values in the same row that are significantly different ($P < 0{\cdot}01$)

Sex	Age class	Habitat		
		Preferred	Peripheral	Combined
Female	Combined	124·8 ± 49·1 (*17*)	262·3 ± 52·3 (*12*)	181·7 ± 37·6 (*29*)
	Subadult	[A] 47·5 ± 29·6 (*4*)	[A] 96·0 ± 18·8 (*3*)	
	Adult	[A]148·6 ± 62·7 (*13*)	[A]317·8 ± 58·7 (*9*)	
Male	Combined	211·4 ± 102·1 (*17*)	394·2 ± 158·1 (*11*)	283·2 ± 87·2 (*28*)
	Subadults	[A]538·0 ± 411·5 (*4*)	[A]931·5 ± 899·6 (*2*)	
	Adult	[A]110·8 ± 35·6 (*13*)	[A]274·8 ± 75·9 (*9*)	
Both sexes	Combined	[B]168·1 ± 55·9 (*34*)	[B]325·4 ± 79·4 (*23*)	231·6 ± 47·0 (*57*)

Paraustrostrongylus trichosuri was recovered from the duodenum of all *T. caninus* (Table 1) and *T. vulpecula* (Table 2) examined. A sex–age interaction ($P < 0{\cdot}05$) was found among *T. caninus*; subadult males, particularly those 3 y old, harboured large *Paraustrostrongylus* burdens (Table 4). Although a feature among subadult males from both habitats, there were more *Paraustrostrongylus* in peripheral-habitat *T. caninus* ($P < 0{\cdot}01$). Among adults *Paraustrostrongylus* burdens were greater ($P < 0{\cdot}01$) in females than in males. Nematodes were coiled tightly around the distal portion of villi (Fig. 5). Swollen enterocytes were evident where alae of *Paraustrostrongylus* made contact with villi; occasionally a mild mononuclear cell infiltration was seen in laminae propriae of affected villi.

Small numbers of *Parastrongyloides trichosuri* were identified by means of microscopic examination of preserved intestinal contents from all *T. caninus* (Table 1)

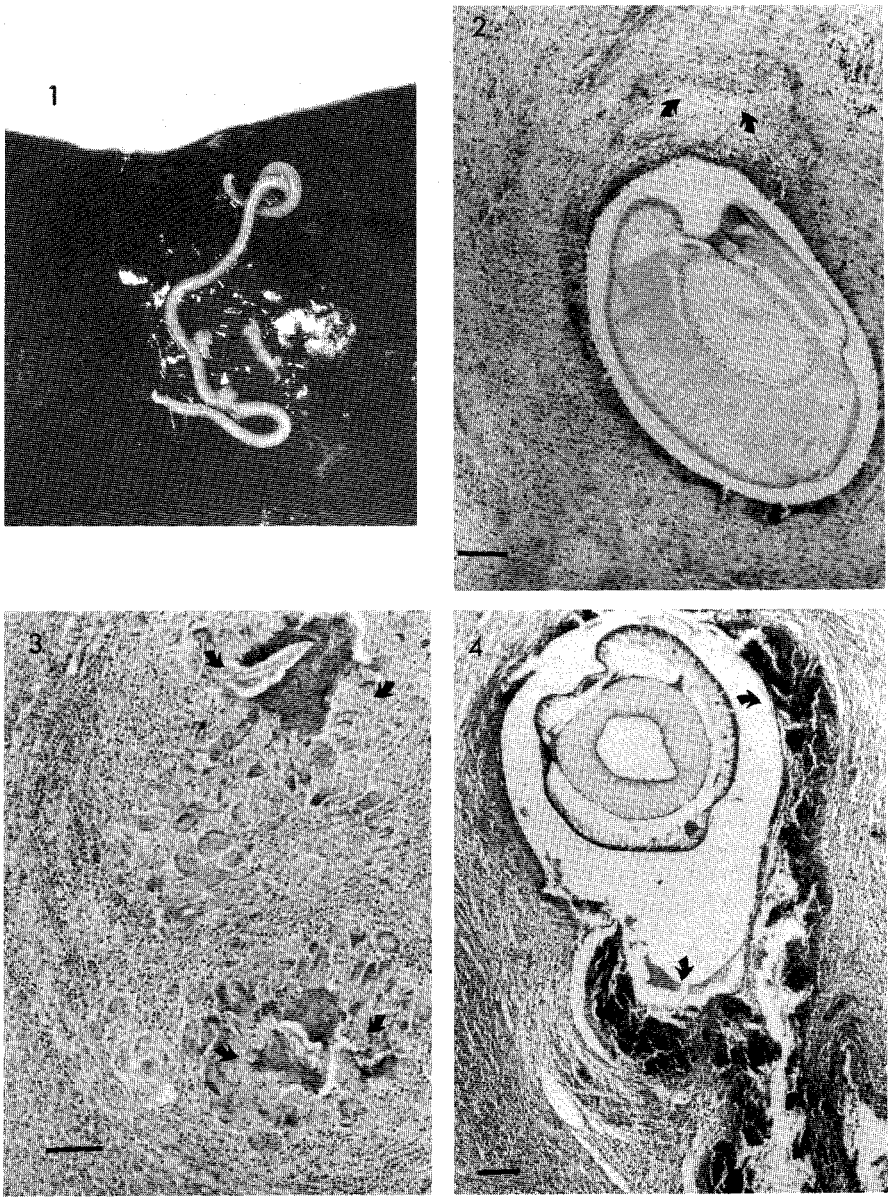

Fig. 1. A third-stage larva of *Amplicaecum robertsi* excised from a subcapsular nodule. Liver of *T. caninus*.
Fig. 2. Focal necrosis and mild eosinophil infiltration (arrows) associated with a migrating *A. robertsi* larva in *T. caninus* liver.
Fig. 3. Focal granulomatous reaction to cellular debris (arrows) in a healing track in liver of *T. caninus*.
Fig. 4. Necrosis and haemorrhage (arrows) in the fibrous walls of a bile duct containing *A. robertsi*.
Figs 2–4 stained haematoxylin and eosin. Bars, 100 μm.

and *T. vulpecula* (Table 2). Section through small nematodes (*Parastrongyloides*) in the laminae propriae of villi in the proximal duodenum caused a mild focal inflammatory reaction.

Prevalence of *Adelonema trichosuri* infection was more common ($P < 0{\cdot}05$) in peripheral-habitat than preferred-habitat *T. caninus* (Table 1), but similar in both sexes and age groups. Burdens were usually light (58%), but moderate (16%) and heavy (26%) burdens were also encountered. Pathological changes were not associated with *Adelonema* infection.

Although the lungs and major bronchioles of *T. caninus* appeared grossly normal, histological examination revealed sections through nematodes, probably *Marsupostrongylus minesi* (Table 1). Mature worms located in alveolar sacs had minimal inflammatory reaction around them (Fig. 6). However, focal interstitial thickening, smooth muscle hyperplasia and occasional granulomatous lesions were associated with lungworm larvae. Lungs from the three *T. vulpecula* were normal.

Table 5. Effects of sex and habitat on plasma corticosteroid levels in adult *T. caninus* at site of final trapping at Clouds Creek, N.S.W.

Values are means ± standard errrors: sample sizes are in parentheses. Superscript letters indicate pairs of values in the same column that differ significantly ($P < 0{\cdot}05$)

Sex	Habitat	Total corticosteroids (ng ml^{-1})	Maximum binding capacity (ng ml^{-1})	Free corticosteroids (ng ml^{-1})
Female	Combined	8·23 ± 0·51 (*22*)	44·86 ± 3·09 (*13*)	1·40 ± 0·12 (*13*)
	Preferred	8·41 ± 0·44 (*14*)	41·36 ± 4·24 (*8*)	^{A}1·57 ± 0·12 (*8*)
	Peripheral	7·93 ± 1·28 (*8*)	50·46 ± 4·18 (*5*)	^{A}1·04 ± 0·20 (*5*)
Male	Combined	7·31 ± 0·42 (*25*)	46·51 ± 1·91 (*18*)	1·25 ± 0·07 (*18*)
	Preferred	7·55 ± 0·50 (*15*)	45·79 ± 2·28 (*13*)	1·25 ± 0·08 (*13*)
	Peripheral	6·95 ± 0·80 (*10*)	48·39 ± 4·17 (*5*)	1·24 ± 0·19 (*5*)
Combined	Preferred	7·96 ± 0·33 (*29*)	44·10 ± 2·08 (*21*)	1·37 ± 0·07 (*21*)
	Peripheral	7·38 ± 0·69 (*18*)	49·43 ± 2·66 (*10*)	1·14 ± 0·13 (*10*)

Section through a filarioid nematode, probably *Sprattia venacavincola*, was found in the portal vein of *T. caninus* (Table 1); it caused marked thickening and oedema of the wall with moderate eosinophil infiltration (Fig. 7). Hepatic vasculitis was evident in six (11%) *T. caninus* and one *T. vulpecula*.

Additional evidence of filarioid infection in *T. caninus* was found on examination of blood films and in sections of the spleen. Sheathed microfilariae with long filamentous tails were found in 14 (25%) *T. caninus*; habitat, sex and age differences were not significant ($P > 0{\cdot}05$). Red nodules 0·5–1·5 cm in size located near the margins of the spleen in 19 (33%) *T. caninus* were granulomatous reactions associated with sequestered microfilariae (Fig. 8). Focal granulomatous splenitis was found in six adult (> 7 y) preferred-habitat *T. caninus* with microfilaraemia, as well as in nine adults (4–13 y) in which no microfilariae were detected. In this latter group, seven were from preferred habitat and two from peripheral habitat. Microfilariae or focal granulomatous splenitis were not detected in *T. vulpecula*.

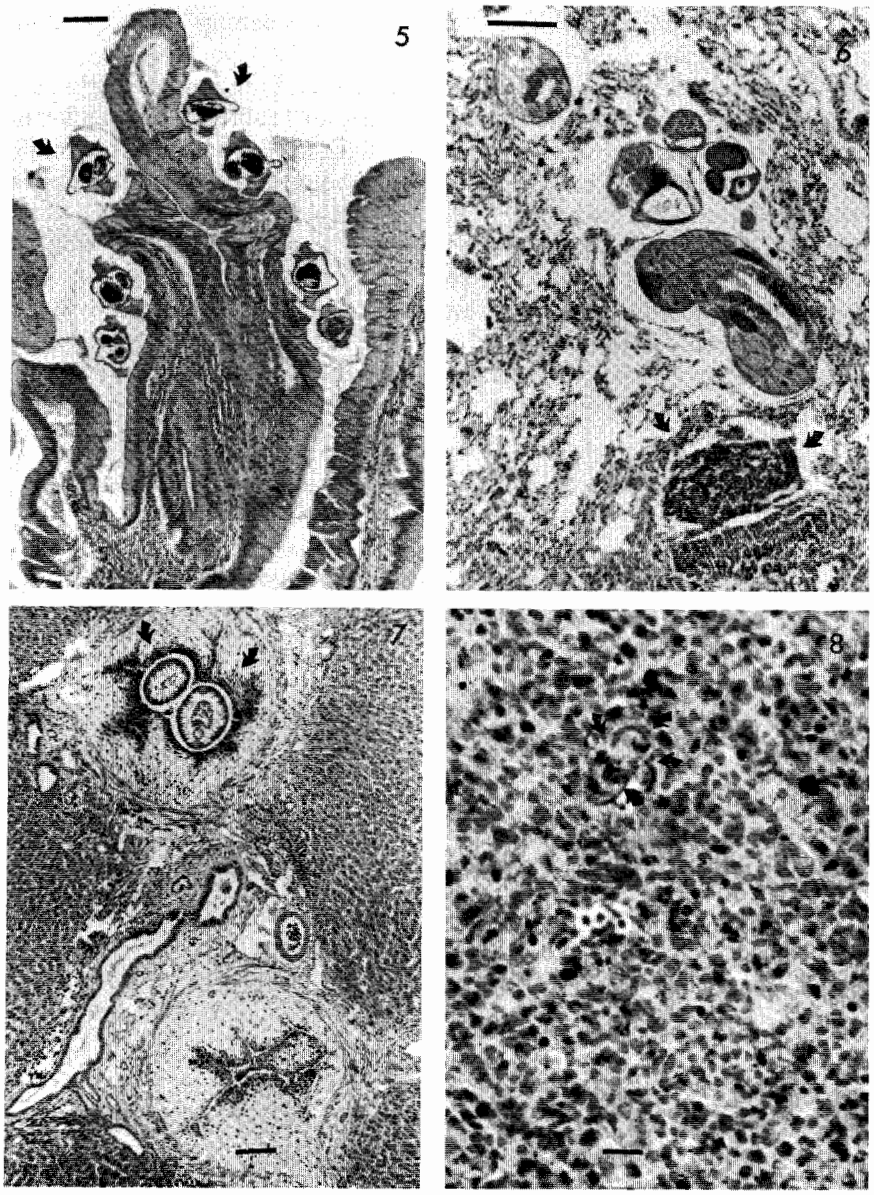

Fig. 5. Longitudinal section through a villus with a *Paraustrostrongylus trichosuri* tightly coiled around it (arrows); duodenum of *T. caninus*. Bar, 100 μm.
Fig. 6. Mature *Marsupostrongylus minesi* in dilated alveolar sacs with adjacent inflammatory focus (arrows); pulmonary parenchyma of *T. caninus*. Bar, 100 μm.
Fig. 7. Phlebitis of a portal vein associated with *Sprattia venacavincola* located in its lumen (arrows); *T. caninus*. Bar, 100 μm.
Fig. 8. Focal granulomatous splenitis associated with sequestered microfilariae (arrows); *T. caninus*. Bar, 10 μm.
All stained haematoxylin and eosin.

Corticosteroid Levels and Adrenal Gland Morphology

Free corticosteroid levels in preferred-habitat females were greater ($P < 0{\cdot}05$) than those in adult females from peripheral habitat (Table 5). Focal nodular hyperplasia of the adrenal cortex was found in five adult preferred-habitat females, and adrenocortical cystic vesicles were noted in three others. There were no correlations between worm burdens and the plasma corticosteroid levels. Focal agonal haemorrhages in adrenal cortices of seven *T. caninus* were attributed to the method of euthanasia. Adrenal glands of the three *T. vulpecula* were normal.

Incidental Pathological Findings

Mild focal interstitial nephritis was found in kidneys of three *T. caninus* and one *T. vulpecula*. Foreign body (plant) penetration in the tongue caused a focal granulomatous lesion in one *T. caninus*. In addition, focal eosinophilic oesophagitis and focal myocarditis were each recorded once in *T. caninus*. None of these lesions was found in *T. vulpecula*.

Discussion

The majority of ectoparasites (Domrow 1958, 1964, 1972; Roberts 1970) and endoparasites (Mackerras 1959; Mawson 1973, 1978; Spratt and Varughese 1975; Beveridge 1976; Spratt 1978) encountered in this investigation have been previously described. However, two mites, two protozoa and three nematodes are new host records for *T. caninus* (Table 1) and *Amplicaecum robertsi* is reported in *T. vulpecula* for the first time (Table 2).

Burdens of *Ixodes holocyclus* on *T. caninus* were lighter than on this host in Queensland (Doube 1979) but the preponderance of immature stages and greater resistance to infestation by *T. vulpecula* were similar. Lesions associated with *Ixodes* spp. and mite infestations were common on *Trichosurus* spp.; irritation and damage to skin has been attributed to dermanyssid mites (Domrow 1979), and rump wear to an allergic reaction to mite infestation (Munday 1978).

Eimerian oocysts in faeces of *T. caninus* were similar in size and morphology to those described in *T. vulpecula* from other localities (Presidente 1981). The sarcocysts in *T. caninus* were described elsewhere (Munday *et al.* 1978). A suitable intermediate host for *Amplicaecum robertsi* in artificial infections (Sprent 1963), the moderate prevalence (53%) of infection in this *T. caninus* population confirms its role as host in the environment. Spratt (1978) described *Marsupostrongylus minesi* from the lungs of *T.(?) caninus*; identification of nematodes in pulmonary sections from *T. caninus* and not *T. vulpecula* suggests that *T. caninus* is the probable host. The nematode in section of *T. caninus* liver was probably *Sprattia venacavincola* (Chabaud and Bain 1976). Unsheathed microfilariae in blood films of *T. caninus* differed from the sheathed larvae of *S. venacavincola* that also lack a filamentous tail (Spratt and Varughese 1975). *Breinlia trichosuri* (Breinl, 1913) occurs in the peritoneal and thoracic cavities of *Trichosurus* spp. (Johnston and Mawson 1940; Spratt and Varughese 1975) and has sheathed microfilariae. This nematode may have been overlooked at postmortem examination.

Habitat Effects on Host Parasites and Condition

Prevalence of three parasites (*Amplicaecum*, *Adelonema* and *Bertiella*) were

greater and tick, mite and *Paraustrostrongylus trichosuri* burdens larger in peripheral-habitat *T. caninus*, but microfilaraemia and splenic granulomatous lesions were more common in preferred-habitat animals. These differences could reflect greater movement or intraspecific contact between animals in peripheral habitat. Subadults disperse earlier in peripheral habitat, but home ranges of adult *T. caninus* in both habitats are of similar size (How 1981; Barnett *et al.* 1982). Analysis of stomach contents revealed that *T. caninus* had different diets in the two habitats (L. Ahern, personal communication 1980) and this probably affected parasite uptake (recruitment).

Climatic and environmental features of these habitats have direct effects on the development and survival of free-living parasitic stages and thus affect the density of the entire parasite populations (Esch *et al.* 1975, 1977). Differential effects of habitat on the survival and accessibility of intermediate hosts for those parasites with indirect life cycles are also involved.

Indirect effects of habitat on parasite burdens are host-mediated and these affect each animal to varying degrees. Animals of lower social status often have heavier worm burdens (Dunsmore 1972; Esch *et al.* 1975); this may also contribute to observed habitat differences. Hyperplastic adrenocortical changes and higher free corticosteroid levels in adult female *T. caninus* from preferred habitat suggested a possible immunosuppressed state that may have allowed more parasites to become established (Esch *et al.* 1975; Stein *et al.* 1976). However, *Paraustrostrongylus* burdens were larger in animals from peripheral habitat.

Condition scores for *T. caninus* in preferred habitat were slightly greater than in those from peripheral habitat, a result that was expected. Pigments in hepatocytes and cortices of kidneys were considered as breakdown products of some staple item in their diet. Since these pigments accumulated with age, between-habitat differences were not expected; some animals assigned peripheral-habitat status had spent their later years in preferred habitat (Barnett *et al.* 1982). When five of these *T. caninus* were included with preferred habitat animals and the data re-analysed, the results were not substantially altered.

Effects of Host Sex and Age on Host Parasites and Condition

A sex–age interaction on *Paraustrostrongylus* burdens in *T. caninus* was demonstrated, burdens were light in animals 1–2 y old, but heavy in two 3-y-old males (Table 4). Since males disperse at this age (How 1978, 1981; Barnett *et al.* 1982) increased burdens may be associated with dispersal rather than age *per se*. Lighter burdens in adult *T. caninus* could indicate the effects of acquired immunity or even changes in diet (Esch *et al.* 1977). Heavier parasite burdens in adult females than in adult males are usually attributed to hormonal influences or reproductive status (Dunsmore and Dudzinski 1968; Esch *et al.* 1977).

Condition scores for female *T. caninus* were greater than those for males. Since most females had pouch young at this time, their greater depot fat stores were probably a reflection of their reproductive status (Barnett *et al.* 1979*b*).

Host age alone had little effect on parasite prevalence or burdens, apart from the observation that older *T. caninus* commonly had splenic lesions. In long-established filarioid infections, sequestration of microfilariae would occur; the worms may have died and microfilaraemia ceased, but splenic nodules would persist.

Host Species Comparisons

Although few sympatric *T. vulpecula* were examined, differences in the ectoparasites and endoparasites, their prevalences and burdens, were evident (Tables 1, 2).

Pigments in liver or kidney were not evident in *T. vulpecula*, which suggested that *T. vulpecula* had different food preferences or metabolism. Mild focal interstitial nephritis in kidneys of both *Trichosurus* spp. resembled lesions caused by *Leptospira interrogans* serovar *balanica in T. vulpecula*. However, antibody to *balcanica*, detected in sera from three *T. vulpecula* at Clouds Creek, was not found in any *T. caninus* sera (Durfee and Presidente 1979).

To conclude, then, differences in parasite prevalences or burdens were found when peripheral-habitat *T. caninus* were compared with preferred-habitat animals at Clouds Creek. Behavioural, haematological, physiological and morphological differences between peripheral and preferred-habitat *T. caninus* have already been reported (Barnett *et al.* 1979*a*, 1979*b*, 1982). Observations on the parasite fauna extend the number of parameters that differ between these *T. caninus* sub-populations in the two habitats.

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