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FACTORS INFLUENCING THE DEVELOPMENT AND CARBOHYDRATE METABOLISM OF *ECHINOCOCCUS GRANULOSUS* IN DOGS

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ABSTRACT: *Echinococcus granulosus* adult worms, 35 days postinfection, were measured for dispersion in the intestines of 10 dogs, a range of morphological characters, and the excreted end products of carbohydrate catabolism following 4 hr incubation in vitro. Most worms were found in the proximal sections of the small intestine, but the pattern of dispersion differed between dogs. Worm development varied both between dogs and between different regions of the small intestine of individual dogs. Overall there was a high level of variability with no simple patterns. Worm metabolism was related to worm development and, also independently, to local population density within the intestine. Larger, more mature worms produced less lactate and, at higher densities, worms tended to produce more acetate and succinate (pathways with a higher energy yield than lactate) and less ethanol. Thus, both more developed worms and high population density are associated with a shift from cytosolic to mitochondrial metabolism. The variation between worm populations along the small intestine along with the observed variation between worm populations from sibling dogs infected with genetically identical parasites suggests that the local host environment has a significant effect on parasite development.

Members of the genus *Echinococcus* (Cestoda: Taeniidae) have a 2-host life cycle. The cystic metacestode develops in the viscera of an herbivorous or omnivorous intermediate host and protoscoleces are produced by clonal multiplication within the cyst. When the cyst is eaten by a carnivorous definitive host, adult worms develop from evaginated protoscoleces within the small intestine. The process of development can be divided into somatic differentiation, consisting of growth and segmentation, and germinal differentiation, involving proglottization and maturation. The occurrence of unsegmented but sexually mature worms and of immature, segmented worms, suggests that somatic and germinal differentiation can take place independently (Smyth, 1971; Smyth and Davies, 1975; Thompson, 1977).

The genus *Echinococcus* is currently classified into 4 species, and a large number of informally designated strains (Thompson and Lymbery, 1988). Results from recent molecular genetic studies suggest that many of these strains deserve specific status, and a taxonomic revision has been recommended for the genus (Lymbery, 1992; Bowles and McManus, 1993; Thompson et al., 1995; Lymbery and Thompson, 1996). The taxon used in this study is the "sheep strain of *E. granulosus,*" which may infect a wide range of intermediate hosts but invariably uses wild, feral, or domestic dogs as definitive hosts.

Previous studies on *E. granulosus* found great variation in establishment, rate of development, intestinal dispersion and metabolism of worms in both naturally and experimentally infected dogs (Thompson, 1977; McManus, 1981; Macpherson et al., 1985; Gemmell et al., 1986; Lymbery et al., 1989; Barriga and Al-Khalidi, 1991). As with most host–parasite systems, the distribution of worms between dogs is overdispersed, with most dogs having no, or only light, infections (Gemmell et al., 1986). The rate of worm development also differs among dogs; Barriga and Al-Khalidi (1991) showed that at least part of this difference could be explained by the genotype of the host. Both Gemmell et al. (1986) and Barriga and Al-Khalidi (1991) found a positive relationship between worm burden and stage of development, in contrast to the crowding effect normally reported in helminths (Keymer, 1982).

Very little work has been carried out on the biochemistry of the adult stage of *E. granulosus.* A study of the uptake and distribution of radiocarbon from a range of exogenous substrates suggested that adults, like protoscoleces, possess the usual glycolytic sequence to the level of phosphoenol pyruvate (PEP), with further metabolism emphasizing succinate production (Bryant and Morseth, 1968). Assays of metabolic end-products released by various samples of adult worms (isolated by R. C. A Thompson) during in vitro incubation have shown that variation in metabolism may be considerable (Bryant and Behm, 1989).

A number of studies have found that *E. granulosus* adults are unevenly dispersed along the intestine, with most in the jejunum and very few in the ileum (Thompson and Eckert, 1983; Macpherson et al., 1985; Gemmell et al., 1986; Lymbery et al., 1989). The usual explanation is that the proximal portion of the intestine provides either a better microtopology for attachment or a physiological environment more suited to the metabolic requirements of developing worms (Thompson and Eckert, 1983; Thompson, 1986, 1995).

In the present study, we examined the effect of intestinal site on worm establishment and on somatic and germinal differentiation. We also measured for worms from different intestinal sites, the relative proportions of different end-products of carbohydrate catabolism released into glucose-supplemented balanced salt solution during incubation immediately after removal from the host, which provides an estimate of the utilization of different pathways of energy metabolism (Bennet et al., 1990).

MATERIALS AND METHODS

Infection of dogs

Single hydatid cysts were obtained from sheep slaughtered at abattoirs in New South Wales, Australia. Protoscoleces were removed, assessed for viability (>80%) by flame cell activity, and used to infect 1 or more dogs. Each dog was infected with 0.3–0.5 ml of protoscoleces, which had been washed in phosphate-buffered saline and packed in a gelatin capsule (0.1 ml is equivalent to approximately 40,000 proto-

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TABLE I. Host and parasite characteristics for experimental infections of dogs with *Echinococcus granulosus*.

Dog	Sex	Age (months)	Breed	Litter	Cyst	Burden
A	M	7	Bull Terrier	1	1	8,833
B	F	7	Kelpie cross	2	2	7,666
C	M	8	Bull Terrier	3	3	7,009
D	M	2	German Shepherd	4	4	25,129
E	M	2	German Shepherd	4	4	63,658
F	F	2	German Shepherd	4	4	61,126
G	M	4	Kelpie cross	5	5	8,817
H	M	4	Kelpie cross	5	6	14,307
I	M	4	Kelpie cross	6	7	27,743
J	M	4	Kelpie cross	6	7	13,484

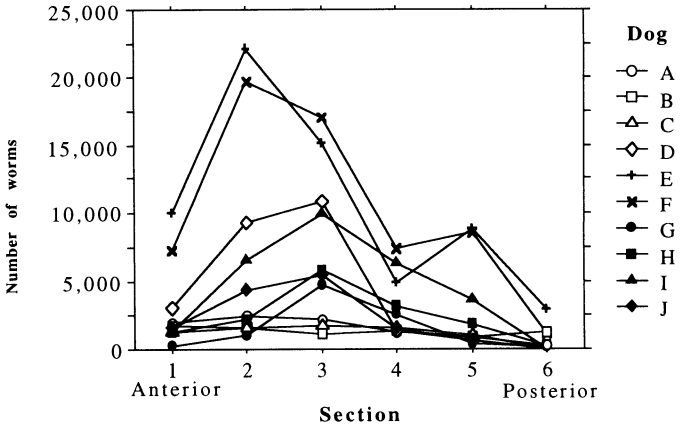

FIGURE 1. *Echinococcus granulosus* worm distribution along the small intestine.

scoleces. Domestic dogs of both sexes and various breeds and ages were infected (Table I). Although we used dogs from the same and different litters, the irregular supply of both dogs and parasite material meant we were unable to design the factorial experiment that would be required to separate the effects of host and parasite genotype on development. Instead, dogs were infected with the same or different material as available, as shown in Table I. All dogs were treated with praziquantel prior to infection to remove any adult tapeworms. After infection, all dogs were housed in similar conditions and maintained on a standard diet of tinned dog meat, dry biscuits, and water ad libitum. The infection was allowed to develop for 35 days, i.e., until just prior to the production of infective eggs.

Sampling of worms

The dogs were fed at the same time once a day except the last morning and were killed by an injection of pentobarbitone sodium at feeding time or up to 2 hr after feeding time. The entire small intestine was removed, opened longitudinally along the line of mesenteric attachment and divided transversely into 6 sections: 5 equal sections from the anterior half of the small intestine and 1 section consisting of the posterior half of the small intestine. Each section was incubated separately in a beaker of Hank's saline (NaCl, 137 mM; KCl, 5 mM; Na_2HPO_4, 0.30 mM; KH_2PO_4, 0.40 mM; $MgSO_4$, 0.40 mM; $CaCl_2$, 0.13 mM; $MgCl_2$, 0.5 mM; $NaHCO_3$, 4 mM) at 37 C for 30 min. The worms were collected and washed in Hank's saline 3 times, once in incubation medium (Hank's saline with glucose, 10 mM; penicillin, 100,000 units/L; and streptomycin, 0.1 g/L; buffered with Hepes, 25 mM, pH 7.5) and then incubated in 1.5 ml incubation medium (containing 22 mM glucose) for 4 hr at 37 C with the flasks open to air. At the end of the incubation period, the medium was removed, centrifuged for 10 min at 20,000 *g* to remove worms, and frozen at −70 C. The exact number of worms in each incubation was determined.

Biochemical studies

The concentration of organic acids and ethanol in the incubation media was determined by high-performance liquid chromatography using a Pharmacia LKB liquid chromatograph equipped with a variable wavelength detector, a differential refractometer, and computing integrator. The column was an Aminex HPX-87H (BioRad) 300 × 7.8 mm with a Cation H Micro-Guard column. A 100-µl sample was injected and eluted with 0.0065 M H_2SO_4 at a flow rate of 0.8 ml/min at 55 C. Organic acids were detected at a wavelength of 210 nm, and ethanol by refractive index detection. Retention times and concentration factors of the organic acids and ethanol were identified by comparison with known standards.

Morphological/developmental studies

Worm numbers in each gut section were counted directly or estimated by subsampling when more than 1,000 worms were present. Total worm length and length of the terminal proglottid were measured as described by Hobbs et al. (1990). Fifty worms from each section were scored for their stage of maturation using the 4 classes described by Kumaratilake et al. (1983): rudimentary genitalia, testes only, testes and rudimentary female genitalia, and expanded uterus; and for their degree of segmentation (as defined by Thompson, 1986): as scolex plus 1, 2, or 3 proglottids. The variables reported here as maturation and segmentation refer to the average of the class scores for 50 worms. The number of hooks, the length of the larval hooks and length of the adult hooks were measured as described by Hobbs et al. (1990). Large hook growth was calculated as (adult hook length − larval hook length)/larval hook length.

Analysis

Heterogeneity in morphological and developmental variables between intestinal sections and among hosts was tested by ANOVA with Scheffe's F-procedure for post-hoc comparisons (testing means that are significantly different from each other). The variance of the number of worms in each intestinal section was used as a measure of worm dispersion for each dog. Principal component analysis was used to investigate patterns of covariation among variables measured for the worms in each section of intestine. Before analysis, the proportions of each excreted metabolic end product were arcsine transformed. Product–moment correlations were obtained among all variables and Bartlett's test of sphericity (which tests for lack of correlation between variables) was used to determine the significance of the correlation matrix. After initial extraction, factors with eigenvalues explaining more than 10% of the variation were retained and rotated to simple structure using the oblique orthotran solution of Hofmann (1978). Whereas this transformation removes the independent (uncorrelated) nature of the factors, it makes the underlying structure clearer by rotating such that for each factor as many variables as possible have either large coefficients or coefficients near zero. Factor loadings greater than 0.3 in the factor–structure matrix were considered to indicate a substantial correlation between variable and factor (Child, 1970).

RESULTS

Intestinal dispersion

Figure 1 shows the dispersion of worms along the small intestine in each experimentally infected dog. Figure 2 shows the same data expressed as proportion of total worm burden in each section, which allows the trends in low worm burden dogs to be seen. There were significant differences in the number of worms within intestinal sections ($P = 0.01$), with most worms located in sections 2 and 3 and fewest in section 6. However, the pattern of dispersion differed between dogs ($P < 0.001$), even for dogs from the same litter and infected with the same parasite isolate. We found no simple relationship between the

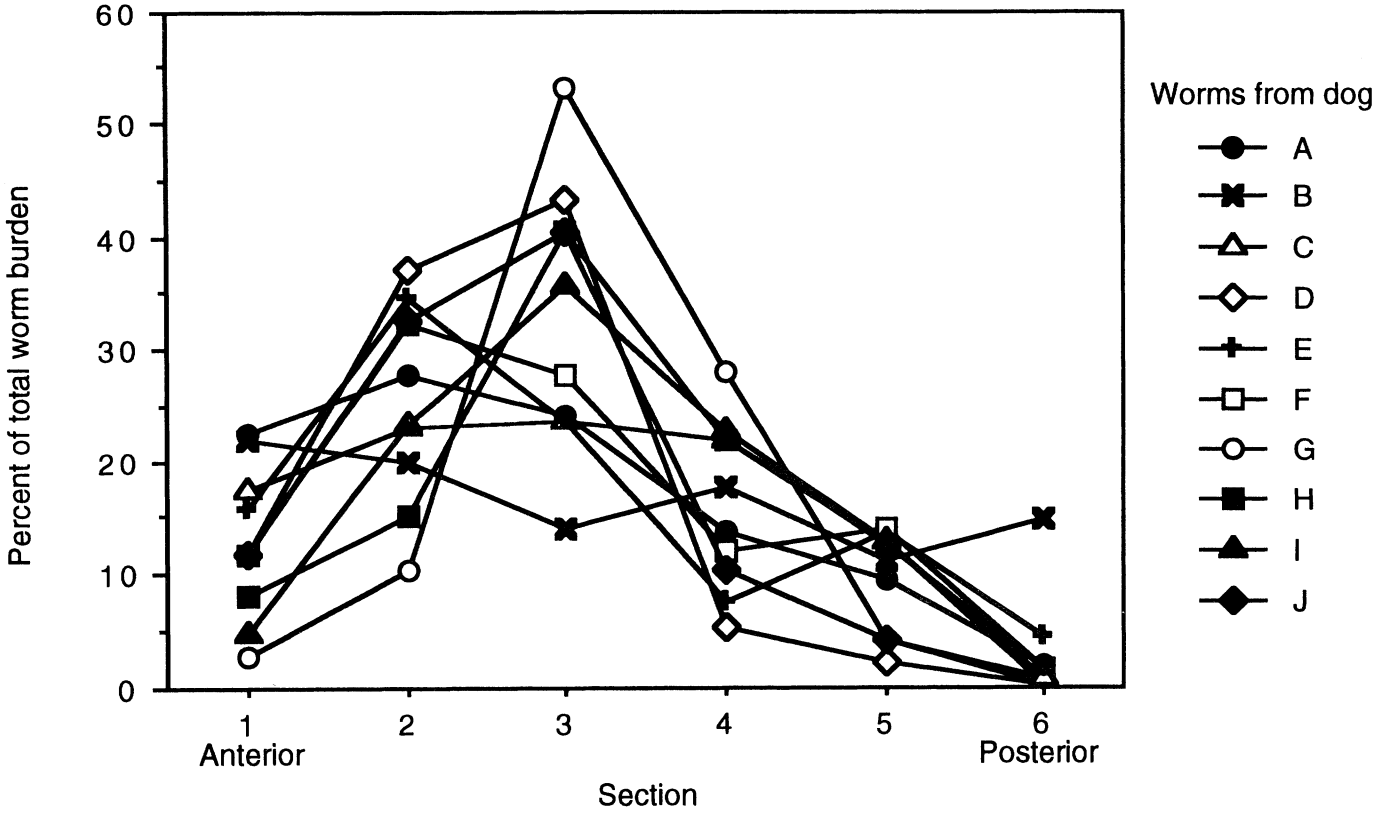

FIGURE 2. *Echinococcus granulosus* worm distribution along the small intestine as proportion of total burden.

degree of dispersion of worms along the small intestine and the total worm burden of each dog (data not shown).

Profile of metabolic end products

Lactate, succinate, acetate, and ethanol were detected in in vitro incubation media from 35-day-old *E. granulosus;* the amounts excreted by worm populations from individual dogs are shown in Table II. Organic acids were detected in all incubation media; ethanol was excreted by worms from some, but not all, dog hosts. As there was considerable variation in size, morphology, and development in the various worm samples, our analysis of the biochemical data is primarily based on qualitative differences (the ratio of end products).

The end-product profiles from worm populations were quite variable. Lactate was the predominant end-product detected in the incubation media from worms isolated from dogs A, B, C, I, and J , whereas succinate was predominant in worms isolated from dogs G and H. Worms isolated from dog E produced equimolar amounts of all 3 organic acids. Ethanol was detected in the largest amounts from worms isolated from dog A, but was a minor end-product, where detected, in other worm populations. Observations made on dogs G–J showed that not all the glucose was utilized during incubation; in vitro incubation medium initially contained 22 mM glucose, 8–10 mM remaining after 4 hr incubation. Metabolic results for dogs D and F were discounted due to a delay in transit before analysis.

Different proportions of end-products were detected from worm samples isolated from different sections of individual dog intestines (Table II). Worms isolated from section 3, or 4 , or both, of the small intestine in general produced proportionally less lactate than the worms from the same infection isolated from sections anterior or posterior to these. Succinate and acetate production were quite variable. Figure 3 shows the ratio of lactate (cytosolic end-product) to succinate plus acetate (mitochondrial end-products) for all the worm samples from each dog. The organic acid ratios varied both between dogs and between sections within a dog. The ratio of organic acid end-products in the media from worms isolated from intestinal section 3 of dogs A, B, C, E, G, and H was lower than that of the worms isolated from sections anterior or posterior. The pattern of end-product ratios for worms isolated from dogs I and J was, however, different. Worms from these 2 dogs were derived from the same cyst.

Morphological/developmental traits

Figures 4, 5, and 6 show how some of the 7 measured morphological and developmental variables vary along the small intestine for each dog. Two-factor analyses of variance indicated significant differences among dogs for all variables. By Scheffes multiple comparison test, worms from dogs D and J were significantly shorter in length (Fig. 4) and had significantly shorter proglottids than worms from other dogs. Worms from dogs B, D, and J were significantly less mature (Fig. 5) and had significantly fewer proglottids than those from other dogs. The large hooks in worms from section 6 grew significantly less than in all other sections (Fig. 6). Hook number and hook length also differed among dogs, but because these variables are largely determined in the intermediate host, they probably

TABLE II. End-products detected in medium after 4 hr incubation in vitro of adult *Echinococcus granulosus* (35 days postinfection) isolated from different gut sections.*

	Gut section (incubations)†	Average number of worms (range)	μmol/ml			
			Lactate (% of total)	Succinate (% of total)	Acetate (% of total)	Ethanol (% of total)
Dog A	1 (3)	140 (104–158)	0.57 ± 0.03 (53)	0.25 ± 0.01 (23)	0.06 ± 0.02 (6)	0.20 ± 0.04 (18)
	2 (4)	163 (145–191)	0.55 ± 0.03 (56)	0.28 ± 0.02 (28)	0.06 ± 0.02 (6)	0.10 ± 0.02 (10)
	3 (4)	147 (124–165)	0.51 ± 0.08 (43)	0.41 ± 0.12 (35)	0.03 ± 0.01 (3)	0.23 ± 0.06 (19)
	4 (4)	144 (129–158)	0.41 ± 0.03 (41)	0.26 ± 0.08 (26)	0.02 ± 0.01 (2)	0.31 ± 0.07 (31)
	5 (4)	138 (120–160)	0.21 ± 0.01 (30)	0.09 ± 0.01 (13)	0.02 ± 0.01 (2)	0.39 ± 0.06 (55)
Dog B	1 (4)	161 (133–178)	0.27 ± 0.01 (47)	0.13 ± 0.01 (23)	0.08 ± 0.04 (14)	0.09 ± 0.01 (16)
	2 (4)	231 (170–261)	0.46 ± 0.03 (59)	0.18 ± 0.01 (23)	0.07 ± 0.02 (9)	0.07 ± 0.01 (9)
	3 (4)	192 (157–214)	0.20 ± 0.05 (31)	0.16 ± 0.02 (25)	0.17 ± 0.04 (27)	0.11 ± 0.05 (17)
	4 (4)	266 (221–313)	0.33 ± 0.04 (52)	0.17 ± 0.02 (27)	0.07 ± 0.01 (11)	0.06 ± 0.01 (10)
	5 (4)	164 (133–202)	0.20 ± 0.06	0.06 ± 0.01	0.02 ± 0.01	No sample
	6 (4)	157 (136–180)	0.26 ± 0.01	0.13 ± 0.03	0.03 ± 0.01	No sample
Dog C	1 (4)	128 (89–197)	0.31 ± 0.04 (68)	0.12 ± 0.04 (26)	0.03 ± 0.01 (6)	0 (0)
	2 (4)	152 (144–160)	0.40 ± 0.05 (56)	0.25 ± 0.04 (35)	0.06 ± 0.01 (9)	0 (0)
	3 (4)	146 (104–187)	0.19 ± 0.03 (46)	0.17 ± 0.04 (41)	0.05 ± 0.01 (13)	0 (0)
	4 (4)	116 (82–152)	0.17 ± 0.04 (55)	0.09 ± 0.03 (29)	0.05 ± 0.01 (16)	0 (0)
	5 (4)	134 (96–173)	0.19 ± 0.02 (53)	0.14 ± 0.02 (39)	0.03 ± 0.01 (8)	0 (0)
Dog E	1 (2)	(185–188)	0.31–0.32 (34–38)	0.32–0.32 (36–38)	0.20–0.27 (24–30)	0 (0)
	2 (4)	160 (143–178)	0.22 ± 0.01 (35)	0.22 ± 0.02 (35)	0.18 ± 0.01 (30)	0 (0)
	3 (3)	142 (124–165)	0.16 ± 0.01 (29)	0.19 ± 0.02 (34)	0.21 ± 0.05 (37)	0 (0)
	4 (4)	174 (147–238)	0.18 ± 0.01 (27)	0.25 ± 0.01 (38)	0.23 ± 0.04 (35)	0 (0)
	5 (3)	190 (136–222)	0.30 ± 0.02 (39)	0.29 ± 0.02 (38)	0.17 ± 0.01 (23)	0 (0)
	6 (1)	110	0.09 (32)	0.08 (29)	0.11 (39)	0 (0)
Dog G	1 (1)	221	0.43 (33)	0.60 (46)	0.27 (20)	0.01 (1)
	2 (3)	212 (135–268)	0.49 ± 0.15 (32)	0.71 ± 0.22 (47)	0.30 ± 0.07 (20)	0.01 ± 0.01 (1)
	3 (4)	235 (211–252)	0.37 ± 0.03 (26)	0.69 ± 0.04 (49)	0.35 ± 0.03 (24)	0.01 ± 0.01 (1)
	4 (3)	207 (204–215)	0.34 ± 0.06 (29)	0.55 ± 0.05 (47)	0.27 ± 0.07 (24)	0 (0)
	5 (1)	245	0.40 (31)	0.60 (47)	0.27 (22)	0 (0)
Dog H	1 (3)	208 (203–217)	0.26 ± 0.03 (31)	0.35 ± 0.06 (41)	0.21 ± 0.02 (25)	0.03 ± 0.02 (3)
	2 (4)	232 (211–250)	0.29 ± 0.02 (22)	0.61 ± 0.01 (46)	0.37 ± 0.03 (28)	0.04 ± 0.02 (4)
	3 (4)	221 (200–232)	0.32 ± 0.01 (21)	0.73 ± 0.08 (48)	0.41 ± 0.03 (27)	0.06 ± 0.04 (4)
	4 (4)	210 (204–216)	0.26 ± 0.04 (27)	0.46 ± 0.08 (47)	0.24 ± 0.03 (25)	0.01 ± 0.01 (1)
	5 (4)	246 (220–272)	0.20 ± 0.05 (21)	0.31 ± 0.10 (32)	0.19 ± 0.05 (20)	0.26 ± 0.18 (27)
	6 (1)	147	0.29 (36)	0.25 (31)	0.24 (30)	0.02 (3)
Dog I	1 (3)	159 (149–172)	0.28 ± 0.06 (56)	0.09 ± 0.04 (18)	0.12 ± 0.03 (24)	0.01 ± 0.01 (2)
	2 (4)	179 (159–190)	0.34 ± 0.03 (52)	0.25 ± 0.04 (38)	0.07 ± 0.01 (10)	0 (0)
	3 (4)	189 (180–194)	0.47 ± 0.07 (48)	0.40 ± 0.07 (41)	0.09 ± 0.02 (9)	0.01 ± 0.01 (2)
	4 (4)	141 (124–157)	0.57 ± 0.20 (55)	0.22 ± 0.05 (21)	0.25 ± 0.11 (24)	0 (0)
	5 (2)	(138–152)	0.31–0.36 (31)	0.43–0.53 (53)	0.16 n = 1 (16)	0 (0)
Dog J	1 (2)	(132–141)	0.35 ± 0.52 (51–53)	0.25–0.36 (35–38)	0.60–0.14 (9–14)	0 (0)
	2 (3)	210 (198–220)	0.50 ± 0.05 (57)	0.29 ± 0.02 (33)	0.07 ± 0.01 (8)	0.01 ± 0.01 (2)
	3 (3)	146 (103–178)	0.35 ± 0.10 (64)	0.14 ± 0.03 (25)	0.05 ± 0.01 (9)	0.01 ± 0.01 (2)
	4 (2)	(111–133)	0.29–0.43 (68–71)	0.06–0.114 (14–17)	0.06–0.08 (13–15)	0–0.01 (0–2)
	5 (1)	111	0.47 (51)	0.01 (1)	0.44 (48)	0 (0)

* Results expressed as $\bar{x}$ ± SE (n ≥ 3) or range (n < 3).

† The length of the intestine was cut into 2 portions of equal length. The anterior half of the intestine was cut into 5 sections of equal length (section 1–5). The posterior half of the intestine was left intact (section 6). Where the number of worms recovered from section 6 was <100 (dogs A, C, G, I, J), end-products from in vitro incubation were below the level of detection in our system.

reflect differences in the isolates used to infect the dogs (Hobbs et al., 1990). For 1 cyst isolate (used to infect sibling dogs D, E, and F), there was a trend for worms from the distal end of the small intestine to have fewer hooks than those from the proximal sections (data not shown).

Analysis of variance also showed significant differences between intestinal sections for worm length, proglottid length, maturity, number of proglottids, and adult hook length. Interpretation was complicated by significant dog by intestinal section interactions for all variables but, in general multiple comparison tests showed that more proximal sections had larger, more mature worms with larger hooks.

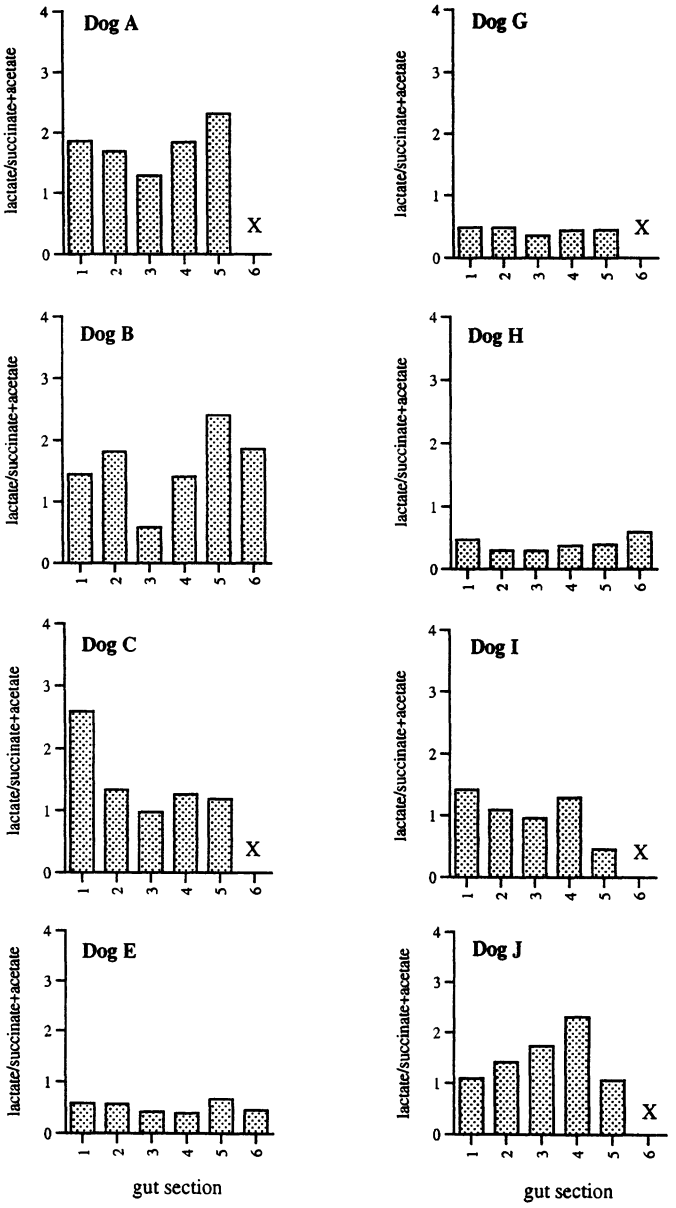

FIGURE 3. Ratio of organic acid end-products (lactate/succinate + acetate) in incubation media from 35-day-old *Echinococcus granulosus* isolated from sequential gut sections of dogs and incubated for 4 hr in vitro. Dogs A, B, C, and E (unrelated) and dogs G and H (siblings) were each infected with material from separate cysts. Dogs I and J (siblings) infected with material from the same cyst. X indicates insufficient worm material for biochemical analysis.

Covariation of developmental and metabolic traits

Many of the traits measured for samples of worms from each intestinal section were significantly correlated (Table III; Bartlett's test of sphericity, $c^2 = 509.2$ $P < 0.001$). Principal component analysis extracted 3 factors that account for 75% of the variation in the data (Table IV). Factor 1, which explains 41% of total variation, had positive loadings for worm length and germinal differentiation traits, indicating that intestinal sections that had large worms also tended to have more mature worms, and negative loadings for number of rostellar hooks and proportional production of lactate. Factor 2, which explains 18% of total variation, had a negative loading for ethanol production and positive loadings for acetate production, worm numbers, and succinate production, indicating that worms from intestinal sections with larger worm populations produced less ethanol in vitro and more acetate and succinate. Factor 3, which explains 16% of total variation, had positive loadings for the 3 rostellar hook traits and succinate production and negative loadings for worm numbers and terminal proglottid length. These 3 factors were not independent (due to orthotran transformation), with intercorrelations between factor 1 and 2 of 0.307, between 1 and 3 of 0.460, and between 2 and 3 of 0.142.

DISCUSSION

This study has demonstrated striking differences between individual dogs in intestinal dispersion, maturation, and metabolism in vitro of adult *E. granulosus*. There were significant differences in worm dispersion even between sibling dogs (dogs D, E, and F, and dogs I and J) that had been infected with the same parasite isolate. It is not possible from this limited study to establish the proportion of the variance attributable to host or parasite genotypes; to achieve this, a far more extensive experimental design than was possible here would be required, with measures to reduce the effects of prior intermediate hosts and of the physiological status of definitive hosts. Unfortunately, almost insurmountable difficulties are associated with experiments that require a sufficient number of dogs and a regular supply of parasites from the same geographical region. We hope that the recently reported rodent models for studying strobilar development (Kamiya and Sato, 1990a, 1990b) may become more widely available and used to investigate the development of *E. granulosus* further (Thompson and Lymbery, 1996).

We found, as have other studies (Thompson and Eckert, 1983; Macpherson et al., 1985; Gemmell et al., 1986; Lymbery et al., 1989), that adult *E. granulosus* are dispersed unevenly along the intestine of dogs, with most worms located in the proximal sections. Lymbery et al. (1989) referred to this uneven dispersion as site restriction. The lack of consistent pattern among dogs and the uneven distribution (overdispersion) within dogs suggest that it is not simply that some zone of the small intestine provides a better environment, e.g., better nutrients (Mettrick and Podesta, 1974), less immune activity, and better attachment, but rather that the worms may be aggregating in response to some local, finer scale microenvironmental effect that is not zone specific. It could simply be that the previously reported ability of adult *E. granulosus* to change position (Thompson, 1986, 1995) and attraction between worms (Lymbery et al., 1989) causes local aggregations. It is possible this may provide some benefits to the worms such as enhancing the local environment by altering nutrient availability or reducing host immune effects.

Dog D (sibling of E and F) and dog J (sibling of I) were both smaller than their sibling(s) and had worms that were significantly smaller and less mature than their siblings despite each group of siblings being infected simultaneously with parasites of the same genotype. These 2 dogs also had half the worm burden of their sibling(s). Thus, it appears that the relative size of the host, which may reflect differences in intestinal physiology, for example, has significant effects on worm establishment and development.

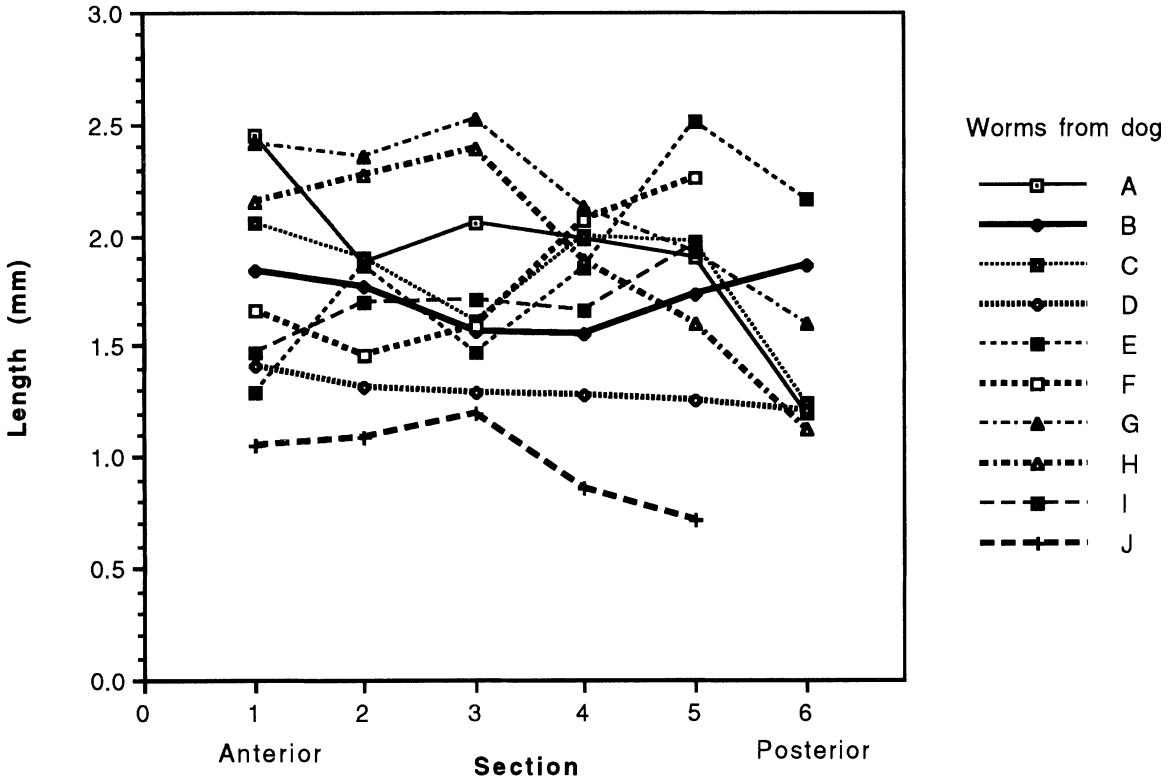

FIGURE 4. Worm length along the small intestine for each dog.

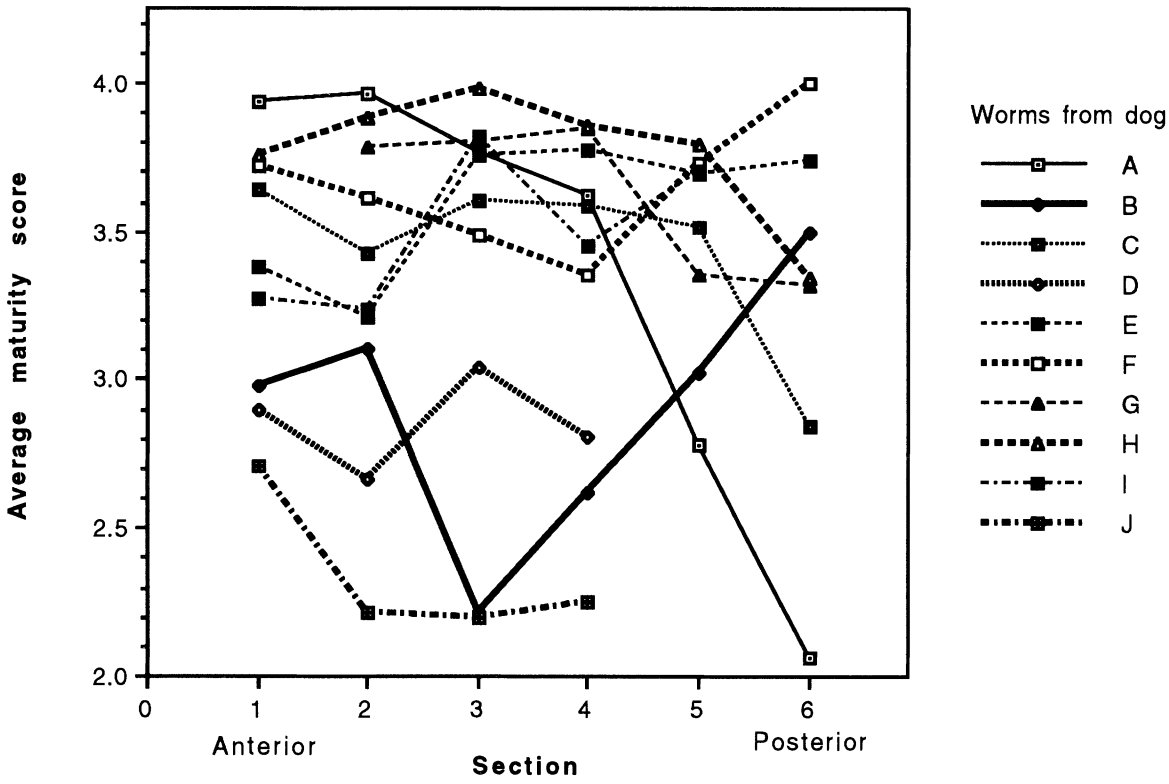

FIGURE 5. Maturity class along the small intestine for each dog.

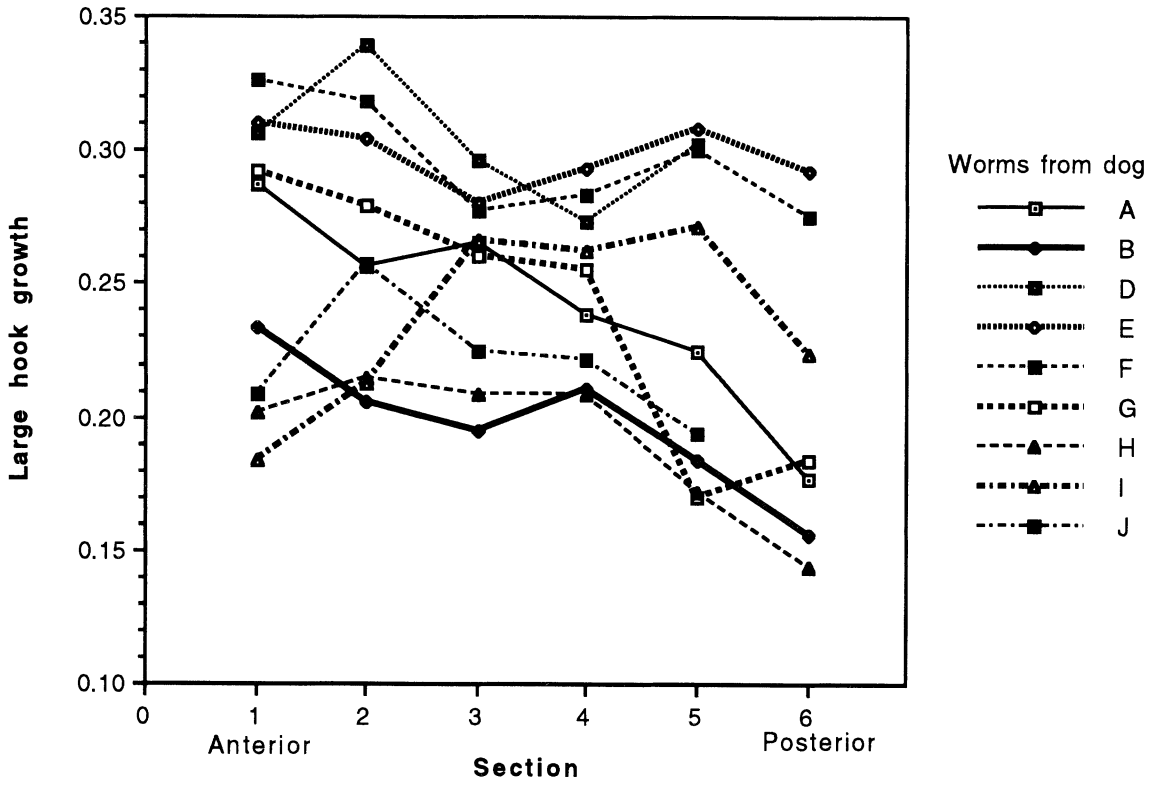

FIGURE 6. Hook growth along the small intestine for each dog.

Factor 3 of the principal component analysis suggests a positive relationship of worm number with proglottid length and a negative relationship with number and size of hooks and succinate production. This could be evidence for partitioning of resources between proglottid and scolex development, but it is not clear why increased worm population density should shift the partitioning in favor of proglottid development. This factor may be related to cyst isolate, due either to parasite genotype or development in the intermediate host. Other than this, there is no evidence of any relationship between worm number and worm development in this study.

Factor 1 of the principal components analysis shows that larger, more mature worms produced proportionately less lactate as a metabolic end-product in vitro. If this proportional decline in lactate production reflects the situation in vivo, it could be evidence of a transition from aerobic to anaerobic energy-producing pathways with maturation in *E. granulosus* adults. A common metabolic pattern among parasitic helminths is that larval and immature adult stages use principally aerobic energy-generating pathways and as they develop into adult worms they tend to use pathways that are maximally adapted to anaerobic energy generation (Bryant and Behm, 1989; Komuniecki and Komuniecki, 1989). A functional citric acid cycle has been demonstrated in protoscoleces of *E. granulosus*

TABLE III. Correlation matrix between all developmental and biochemical variables for adult *Echinococcus granulosus* worms.

	Number of worms	Length	Proglottid length	Maturity	Segmentation	Hook number	Adult hook	Larval hook	Lactate	Succinate	Acetate	Ethanol
Number of worms	1.000	−0.017	0.042	0.109	0.161	−0.043	−0.161	−0.439	−0.076	0.176	0.309	−0.425
Length of worm	−0.017	1.000	0.964	0.703	0.691	−0.252	0.578	0.302	−0.506	0.412	0.068	0.102
Proglottid length	0.042	0.964	1.000	0.674	0.691	−0.352	0.444	0.167	−0.436	0.304	0.086	0.081
Maturity	0.109	0.703	0.674	1.000	0.972	−0.073	0.588	0.346	−0.540	0.528	0.247	−0.119
Segmentation	0.161	0.691	0.661	0.972	1.000	−0.079	0.570	0.280	−0.475	0.507	0.245	−0.136
Hook number	−0.043	−0.252	−0.352	−0.073	−0.079	1.000	0.224	0.352	0.070	0.141	−0.104	−0.099
Adult hook length	−0.161	0.578	0.444	0.588	0.570	0.224	1.000	0.830	−0.472	0.599	0.104	−0.066
Larval hook length	−0.439	0.302	0.167	0.346	0.280	0.352	0.830	1.000	−0.411	0.439	0.071	0.095
Lactate production	−0.076	−0.506	−0.436	−0.540	−0.475	0.070	−0.472	−0.411	1.000	−0.567	−0.540	−0.016
Succinate production	0.176	0.412	0.304	0.528	0.507	0.141	0.599	0.439	−0.567	1.000	0.373	−0.537
Acetate production	0.309	0.068	0.086	0.247	0.245	−0.104	0.104	0.071	−0.540	0.373	1.000	−0.611
Ethanol production	−0.425	0.102	0.081	−0.119	−0.136	−0.099	−0.066	0.095	−0.016	−0.537	−0.611	1.000

TABLE IV. Factor structure matrix, showing weighting of developmental/biochemical variables for each factor from principal components analysis (oblique solution). Significant values are in bold.

	Factor 1	Factor 2	Factor 3
Number of worms	0.044	**0.693**	**−0.460**
Length	**1.056**	−0.223	−0.176
Proglottid length	**1.086**	−0.205	**−0.355**
Maturity	**0.864**	0.093	0.003
Segmentation	**0.858**	0.118	−0.047
Hook number	**−0.616**	0.118	**0.828**
Adult hook length	**0.427**	−0.066	**0.663**
Larval hook length	0.093	−0.204	**0.909**
Lactate production	**−0.539**	−0.217	−0.176
Succinate production	0.248	**0.521**	**0.426**
Acetate production	0.034	**0.790**	0.000
Ethanol production	0.277	**−0.925**	−0.085

(McManus and Smyth, 1978, 1982), but it is not known whether this is the case in adults, or whether oxidative phosphorylation utilizing oxygen as terminal electron acceptor plays any role in their metabolism. We did not measure citric acid cycle activity or cyanide-sensitive respiration and so could not estimate the contribution of fully oxidative metabolism to energy generation in adult *E. granulosus* under our incubation conditions. The large amount of glucose consumed relative to the amount accounted for by the end products measured suggests that glycogen synthesis, and perhaps fully oxidative metabolism, may be significant.

Factor 2 of the principal components analysis shows that in intestinal sections with a higher density of worms, more acetate and succinate and less ethanol were produced. Ethanol was produced only by worm populations from a small proportion of dogs. The origin of ethanol in *E. granulosus* is not known, but it would normally be expected to be derived from decarboxylation of pyruvate and would yield the same amount of ATP/mol glucose as lactate production. Thus, crowding of the worms coincides with a shift to the production of succinate and acetate in those worms producing ethanol. Whether such a shift might occur in vivo cannot be determined from these experiments as the incubations did not closely replicate in vivo conditions, being performed open to air and not under a more physiological gas phase that would include higher CO_2 and possibly lower oxygen concentrations. If such a shift does occur in vivo, however, it could reflect a local decrease in pH and increase in CO_2 tension in regions of high worm population density, as has been shown in the small intestine of rats infected with *Hymenolepis diminuta* (Podesta and Mettrick, 1974). It could also be that competition for substrates in the more crowded situation causes the worms to switch to succinate and acetate production, which, if it occurs by pathways demonstrated in other helminths, would yield more ATP/mol glucose than ethanol production. However, in interpreting this result, the confounding effects of active site selection must also be taken into account; the higher population density may be due to active selection of worms for a favorable intestinal environment, which itself could influence the shift in metabolism. Previous studies (Gemmel et al., 1986; Barriga and Al-Kalidi, 1991) have reported a "reverse crowding effect" (i.e., more developed worms at higher population densities) in *E. granulosus,* which would suggest benefits from increased density. In the current study, however, we found no relationship between population density and length or maturity of worms.

The host's immune response could also influence the metabolism of *E. granulosus* adults. *Hymenolepis diminuta* adults removed from rats that had been previously infected with *Nippostrongylus brasiliensis* produced mainly lactate in vitro. They were also smaller and their survival was lower than worms from naive rats, which produced succinate and acetate in vitro (Bennet et al., 1993). This suggests that nonspecific host immune responses can affect the physical and metabolic development of tapeworms. Such immune responses may be very local. Deplazes et al. (1994) found that Peyer's patches showed greater responsiveness to worm antigens than that of cells from peripheral blood and mesenteric or popliteal lymph nodes. The production of IgM has been shown to vary quantitatively along the intestine of dogs, with levels in the duodenum and jejunum greater than those in the ileum (Willard et al., 1978). A limitation of our study was that we were able to examine only 1 cohort of adult worms of a single age. In nature, successive ingestion of cysts may occur, resulting not only in infections of mixed genotype and age, but also an increased likelihood of effective host immune responses; this issue should be considered in future studies.

In the present study, we have found correlations between worm density, rate of development, and the utilization of different metabolic pathways by *E. granulosus* in its definitive host. Although the precise causal relationships underlying these correlations are still to be determined, our results suggest that both high worm densities and more rapid somatic and germinal differentiation are independently associated with a transition from cytosolic to mitochondrial energy metabolism. We hypothesize that differences in worm density along the intestine arise initially through attraction of worms to other worms or to particular microenvironmental sites. Although these high density sites may initially promote rapid development as the worms grow, competitive interactions or perhaps enhanced local immune responses by the host inhibit development and promote more energetically efficient mitochondrial metabolism.

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