Ancylostoma ceylanicum in the hamster: observations on the host–parasite relationship during primary infection

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SUMMARY

The course of primary infection with a hamster-adapted strain of *Ancylostoma ceylanicum* was studied in inbred DSN and randomly bred WO/GD and WO/CR hamsters. Infective larvae were administered orally and began to develop in the small intestine without embarking on a tissue migration. Only the occasional larva was detected in other organ sites. It was concluded that the developing larvae moulted on days 3–4 and again to the pre-adult stage about 9–11 days post-infection. Worm burdens in infected hamsters were stable for at least 11 weeks after infection. There was no sudden expulsive phase and some adult worms survived for over 200 days. Overall the sex ratio of worms in groups of hamsters killed concurrently was about 50% although occasionally the ratio was biased in favour of one sex in individual animals. The blood packed cell volume (PCV) was significantly depressed 2 weeks following infection and continued to decline until a point of stability was achieved 4–5 weeks post-infection. The PCV subsequently remained depressed throughout the period of observation. Infected hamsters lost weight if kept in groups, but not when housed in separate cages. Groups of animals which lost weight did not recover to normal values within 11 weeks of infection. It is suggested that this model of hookworm infection has scope for exploring aspects of the host–parasite relationship which the canine models cannot fulfill adequately.

Key words: *Ancylostoma ceylanicum*, infection model, host–parasite relationship.

INTRODUCTION

Human hookworm infections are still widely distributed in tropical and subtropical regions and constitute a significant source of morbidity and malaise among indigenous populations (Gilles, 1985; Behnke, 1987). The principal species infecting man are *Necator americanus* and *Ancylostoma duodenale*, both of which are considered to be anthropophilic and are essentially confined to human hosts in nature. However, the predominantly canine and feline parasite *A. ceylanicum* has also been shown to be infective to man, having been identified from naturally exposed individuals (Anten & Zuidema, 1964; Chowdurry & Schad, 1972) and patent infections in experimentally infected volunteers (Yoshida, Okamoto & Chui, 1971; Carroll & Grove, 1986). A strain of this species has also been adapted for passage through hamsters (Ray, Bhopale & Shrivastava, 1972) enabling the parasite to be maintained in a relatively inexpensive and experimentally manipulable host.

Nevertheless, apart from the studies of Indian workers this model of hookworm infection has not been widely exploited to study aspects of the host–parasite relationship. Recently, infective larvae of the hamster-adapted strain of *A. ceylanicum* were obtained from India and preliminary experiments in syngeneic DSN hamsters indicated differences between our results and those reported by previous workers (Menon & Bhopale, 1985; Visen, Katiyar & Sen, 1984). In this paper we present the results of our observations on the time-course of primary infections in hamsters and on the accompanying changes in host weight and blood packed cell volumes, reflecting some pathological consequences of infection.

MATERIALS AND METHODS

Animals

Syngeneic DSN hamsters were bought in at 3–4 weeks of age from Shamrock Farms. Randomly bred WO/GD and WO/CR hamsters were bought from Wrights of Essex Ltd. Animals were infected at 8–12 weeks of age and were kept under standard animal house conditions with food and water provided *ad libitum*.

Parasite

Infective larvae of the hamster adapted strain of *A. ceylanicum* were obtained from Dr Rajasekariah, Hindustan Ciba–Geigy Ltd, Bombay, India in 1984, since when the parasite has been maintained through ten generations in our laboratory.

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Infection of hamsters

Infective larvae of the *Ancylostoma* species have the capacity to establish in the host following oral or percutaneous infection (Yoshida *et al.* 1971). It was therefore necessary to compare the routes of infection to determine which would yield the highest and most consistent worm burdens. Three methods of infection were used. Intragastric (gavage): animals anaesthetized with Trilene (ICI) were inoculated directly into the stomach lumen via a blunt-ended 21 G needle with infective larvae suspended in no more than 0·5ml of water. For cheek pouch inoculations larvae were suspended in 20–40 μl of water and placed into the cheek pouch of an anaesthetized animal using a Gilsen pipette. Percutaneous infections were carried out according to the method of Behnke, Wells & Brown (1986).

Faecal egg counts

Faecal egg counts were carried out as described by Behnke & Parish (1979). Samples of 1 g were taken from pooled faeces collected overnight and 4 counts were made in McMaster Egg Counting Chambers on each separate sample.

Autopsy

Animals were killed by an overdose of anaesthetic and the various organs together with the intestine were removed immediately. Adult worms were picked out individually with the aid of a dissecting microscope. L_3 and L_4 stages were recovered using a modified Baermann technique.

Measurement of worms

All the worms were collected following autopsy, pooled and preserved in formalin/ethanol. A sample of 10 worms of each sex was then chosen randomly for drawing with the aid of a camera lucida and these drawings were measured with a bit-pad digitizer linked to an Apple PC.

Measurement of host parameters

Hamsters were individually weighed at weekly intervals using a top pan balance. Blood samples were obtained by bleeding from the retro-orbital sinus under trilene anaesthesia. Blood was collected into 50 μl heparinized capillary tubes, centrifuged for 5 min in a Hawksley Haematocrit centrifuge and the packed cell volume (PCV) was measured.

Statistical analysis of results

The results are presented as mean values ± standard error (S.E.M.) for groups of animals undergoing uniform treatment. Differences between groups were analysed using standard parametric one-way analysis of variance (ANOVA) or the non-parametric Kruskal–Wallis one-way analysis of variance by ranks test (KR ANOVA). Where appropriate the correlation coefficient was calculated and the data for experimental groups, followed over time, were compared at set time-points using the Mann–Whitney *U* test (Sokal & Rohlf, 1969). The Elsevier-Biosoft PC software package Tadpole (Caradoc-Davies, 1985) was used to analyse the data.

RESULTS

Route of infection

Two separate experiments were carried out in which groups of 6 hamsters were infected with 50 L_3 by cheek pouch, intragastric or percutaneous routes. Three weeks later the animals were killed for worm counts. As can be seen from Table 1, despite the variation in worm burdens between groups, the intragastrically administered larvae gave the heaviest and most consistent worm counts.

Route of migration

It was necessary to establish whether the parasites began to grow in the intestine on arrival as L_3 or whether a tissue migration was embarked upon as described in mice infected with the dog strain of *A. ceylanicum* (Carroll, Grove, Dawkins, Mitchell & Whitten, 1983). Groups of 4 hamsters were infected intragastrically with 220 L_3 and killed on days 2, 4, 5, 6, 7, 9, 10, 11, 15 and 17 post-infection (p.i.) In all cases the majority of worms were recovered from the small intestine. Examination of the lungs and liver did not yield parasites at any stage. A single larva was recovered from the abdominal muscle of 1 animal on day 2. Two others each had 1 L_3 in the large intestine and 2 animals had 1 larva in the caecum on day 4. Otherwise the parasites were only recovered from the small intestine.

Growth and moulting

The length of larvae and subsequently adult male and female worms were measured and the results are presented in Table 2. The data suggest that the L_3–L_4 moult occurred on days 3–4 and the L_4–pre-adult moult on days 9–11.

Faecal egg counts and the duration of infection

The time-course of infection was monitored in several experiments in DSN hamsters of both sexes and in the outbred WO/CR and WO/GD strains. The results are presented in Fig. 1. According to weekly egg counts the earliest day on which eggs were

Table 1. Comparison of different routes of infection with *Ancylostoma ceylanicum* in hamsters

(All the animals were killed for worm counts 21 days after infection with 50 L_3 of *A. ceylanicum*.)

Experiment and route of infection	No. of hamsters	Mean number of worms recovered ±S.E.M.*		
		Male	Female	Total
1A Cheek pouch	5	11·4±4·2	15·8±5·7	27·2±9·9
1B Intragastric	6	21·5±5·3	16·3±4·7	37·8±9·9
1C Percutaneous	6	0	0	0
2A Cheek pouch	6	3·3±0·5	3·0±0·8	6·3±1·2
2B Intragastric	6	4·2±0·9	4·3±1·0	8·5±0·8
2C Percutaneous	6	0·5±0·2	2·2±1·0	2·7±1·2

* Statistical analysis of results. KW. ANOVA: Exp. 1, $H = 11{\cdot}129$, $P < 0{\cdot}01$; Exp. 2, $H = 66{\cdot}320$, $P < 0{\cdot}01$. Mann Whitney U test: Exp. 1, A *vs.* B, P = NS; Exp. 2, B *vs.* C, $P < 0{\cdot}005$; Exp. 2, A *vs.* C, $P < 0{\cdot}05$.

Table 2. The growth of *Ancylostoma ceylanicum* during the first 17 days after infection

Day after infection	Mean length (mm) ±S.E.M.*		Observations
	Male worms	Female worms	
2		0·62±0·03	Exsheathed L_3
4		0·99±0·05	L_4; buccal capsule, evidence of sexual differentiation
7	1·8±0·09	2·02±0·13	L_4; sexes distinguishable; male bursa still ensheathed
9	2·8±0·09	2·7±0·13	Male bursa still ensheathed
11	4·0±0·16	3·8±0·16	Pre-adult stage; male bursa now clearly free; worms feeding on blood; females, no fertile eggs
15	5·04±0·19	5·2±0·19	Female worms fecund
17	4·5±0·19	5·1±0·19	

* Ten worms were measured on days 2 and 4. Thereafter 10 male and 10 female worms were measured on all occasions.

detected in the faeces was 14 in male DSN, 12 in female DSN, 14 in male WO/CR, 21 in female WO/CR and 14 in male and female WO/GD. The pattern of faecal egg output was broadly similar in all of the experiments. Thus peak egg production of 9000–11000 eggs/g (e.p.g.) of faeces was usually recorded between 21 and 28 days p.i. This was followed by a plateau of stable egg output with little variation but of varying duration. In some animals egg counts were noticeably reduced by 40–60 days, in others, egg counts did not decline until 100 days p.i. However, egg counts did not decline to zero once the phase of reduction had been initiated. A trickle of egg output persisted for some time, as is seen in Fig. 1. In hamster AcE (Fig. 1A), egg production continued until day 192 and at autopsy on day 216 p.i., 1 male and 1 female worm were recovered.

There was no noticeable difference between the overall pattern and intensity of faecal egg recovery between male and female hamsters. This contrasts with *N. americanus* in hamsters where male animals, given doses of L_3 comparable to those given to females, produce significantly more parasite eggs in their faeces despite similar worm burdens (Behnke & Pritchard, 1987).

Time-course of infection as monitored through worm counts

Changes in the number of parasites were monitored in 3 separate experiments over varying periods of time. In the first two experiments (Fig. 2A, B) there were no significant changes in parasite burdens over the period of observation. Although worm burdens

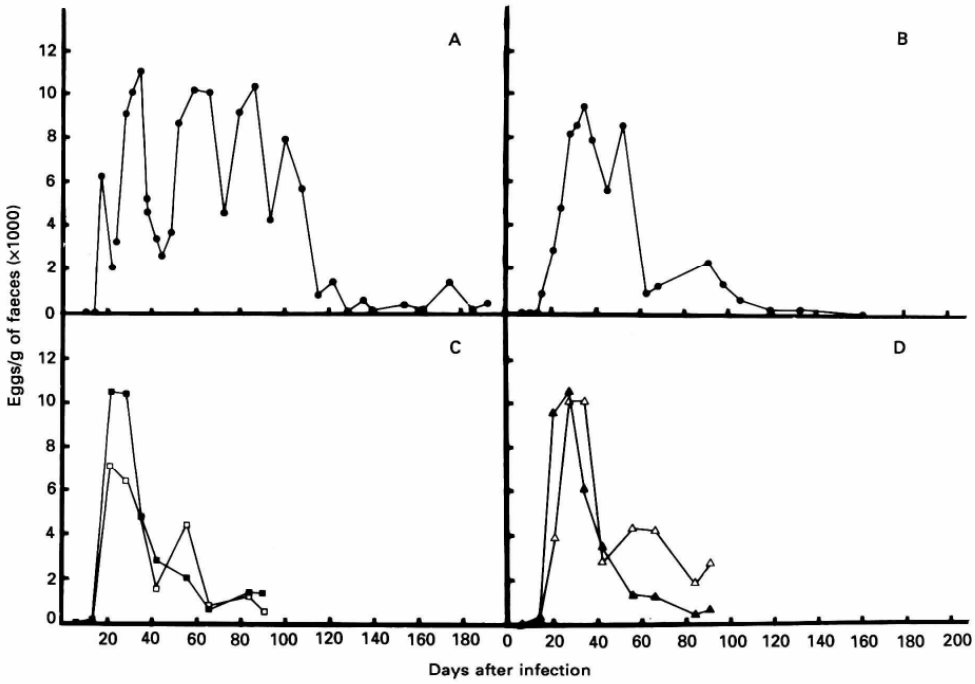

Fig. 1. Faecal egg counts during the course of infection with *Ancylostoma ceylanicum* in hamsters. (A) A single male DSN hamster (AcE). (B) A group of 6 male DSN hamsters. (C) Open symbols, male and closed symbols, female WO/CR hamsters. (D) Open symbols, male and closed symbols, female WO/GD hamsters. All the animals were given approximately 50 L_3.

were numerically and significantly reduced by week 10 in Exp. 3 (Fig. 2C; linear correlation coefficient $= -0{\cdot}455$ (20·7%), D.F. $= 53$, $T = -3{\cdot}719$, $P < 0{\cdot}038$; ANOVA, $F = 4{\cdot}093$, $P < 0{\cdot}0038$) no sudden expulsion was observed. In addition to the experiments which have been illustrated in Fig. 2, we have kept records of the faecal egg counts of animals used to passage the parasite in our laboratory over the last 4 years. Animals killed when faecal egg counts were no longer detectable, usually 20–30 weeks after infection, frequently still harboured adult worms although the worm burdens generally consisted of no more than 2 or 3 parasites, suggesting that worm loss had taken place slowly.

Sex ratios

The percentage of worms of each sex recovered at autopsy was approxmately 50%. This was true throughout the time-course of an infection; for example, in Exp. 2 (Fig. 2B) the mean percentage of male worms was 47, 53, 47, 55 and 46 on days 14, 21, 28, 42 and 63 p.i. Despite the overall stability of the sex ratio, as determined from groups of animals, there was considerable variation in the sex ratios of worms between individual animals; for example, on day 21 p.i. 1 animal contained 7 male and 3 female worms (70% males) and on day 28 p.i. 1 hamster harboured 4 male and 8 female worms representing 33·3% males.

Effect of A. ceylanicum *on PCV and body weight*

The PCV of infected animals was noticeably depressed 2 weeks p.i. and reached its lowest at 35 days p.i., after which a period of relative stability ensued in moderately infected animals (10–30 worms; Fig. 3). When infected hamsters were kept in groups ($n = 8$) weight loss was first detected 14 days p.i. but a significant reduction in weight was not apparent until day 20 (Exp. 1, Fig. 4A; $P = 0{\cdot}04$) or day 21 (Exp. 2, Fig. 4B; $P = 0{\cdot}04$). There was a significant difference on all subsequent time-points compared in both experiments. A state of relative stability was observed after day 28 p.i. in animals given 50 L_3, the hamsters neither gaining nor losing more weight. However, the more heavily infected animals in Exp. 2 (Fig. 4B) continued to lose weight and the apparent stability after day 21 was caused by the removal of those animals which began to show severe symptoms of disease and consequently had to be culled.

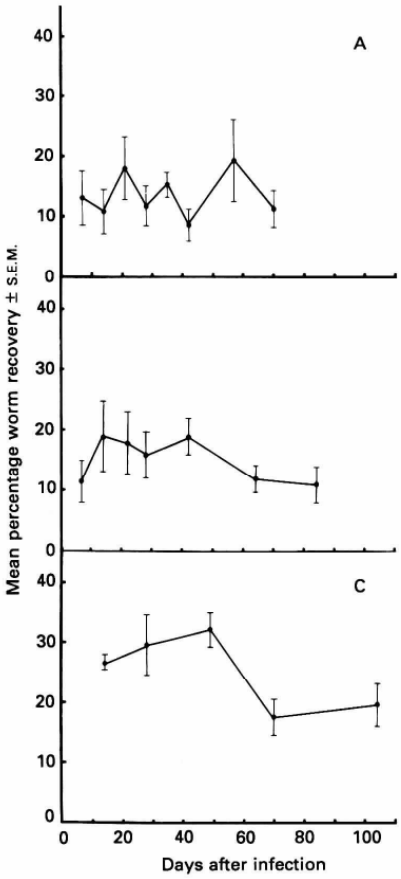

Fig. 2. Worm burdens during infection with *Ancylostoma ceylanicum* in male DSN hamsters. The data are presented as the mean percentage ± S.E.M. of the inoculum administered. (A) Exp. 1 in which groups of 4 hamsters were infected with 55 L_3 and were killed on the days shown. (B) Exp. 2 ($n = 4$) 50 L_3 were given. (C) Exp. 3 [$n = 9$ (weeks 2 and 10), 10 (weeks 4 and 7), 8 (week15)], 54 L_3 were given.

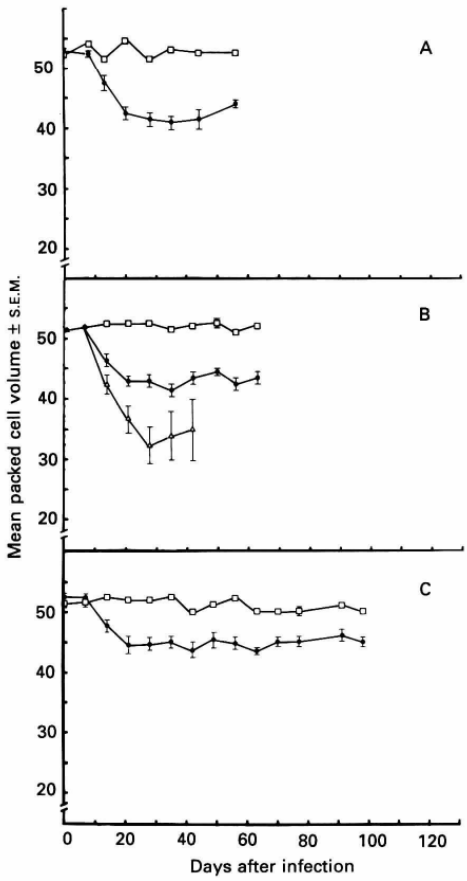

Fig. 3. The packed cell volume of blood samples taken throughout the course of infection with *Ancylostoma ceylanicum* in male DSN hamsters. (A–C) Correspond to the experiments explained in legend to Fig. 2. (□) Control animals; (●), infected animals; (△), an additional group of 8 hamsters given 100 L_3. Where no S.E.M.s are evident for the control groups on this figure, the values calculated did not extend beyond the margins of the symbol used to represent the mean. The data were compared using the Mann–Whitney U test. Infected hamsters had significantly reduced PCV on all occasions after week 1 p.i.

DISCUSSION

The experiments reported in this paper confirm and extend the findings of previous reports on the host–parasite relationship of *A. ceylanicum* in hamsters. It is clear that the parasite is infective to hamsters and can mature to produce fecund parasites which cause chronic infections in the host.

Initially it was important to investigate the variables in the experimental protocol which influence the outcome of infection. Three routes of infection were compared in order to determine the optimal procedures for establishing adult worms. Caroll & Grove (1984, 1986) used percutaneous infection exclusively in their experiments with *A. ceylanicum* in dogs and human volunteers. However, Yoshida *et al.* (1971) had shown earlier that more larvae matured in man following oral infection, and our results support these findings in that marginally fewer parasites established when hamsters were infected into the cheek pouch and markedly fewer following percutaneous infection. The reasons for reduced parasite establishment following percutaneous infection are not known. Presumably such larvae undergo migration through the lungs and respiratory tract before localizing in the intestine. Host effector mechanisms may prevent some of the larvae completing this journey, as has

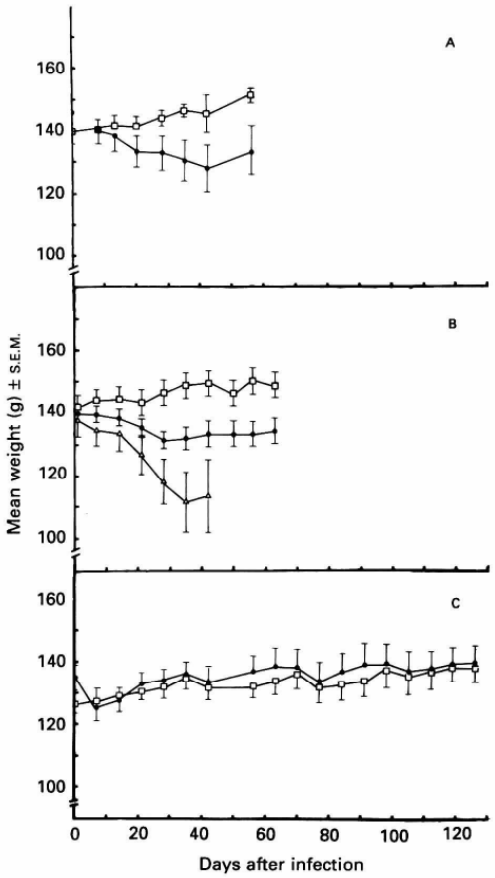

Fig. 4. Changes in the mean body weight of male DSN hamsters during the course of infection with *Ancylostoma ceylanicum*. (A–C) Correspond to the experiments explained in legend to Fig. 2. Symbols as in Fig. 3. The animals in Exps 1 and 2 (Fig. 3A, B) were kept in groups of 8 to a cage. Comparison of the data using the Mann–Whitney *U* test showed that there was a significant reduction in weight in week 2 and on all subsequent occasions. In Exp. 3 (Fig. 4C), both control and infected animals were housed in separate cages ($n = 10$ for both groups). There were no significant differences between the weights of control and infected groups in Exp. 3.

been shown to occur in the case of *N. americanus* in mice (Wells & Behnke, 1988). Despite finding an occasional larva in the musculature we concluded that significant tissue migration was unlikely to have occurred following oral exposure to the parasite. It is possible that some larvae penetrated the mouth, intestine or perianal skin and entered the tissues. If this is the case, the majority of such larvae failed to return to the gut as no increase in worm burden was recorded and the established parasites grew uniformly until patency. However, penetrating larvae may not have completed their migration, becoming arrested in the tissues and, because of the small numbers involved in relation to the total inoculum, examination of the liver and musculature revealed only the one parasite. Overall, our results therefore concur with the report by Ray *et al.* (1972) but contrast with studies using *A. caninum* and *A. duodenale*, both of which undergo tissue migration and arrested development in abnormal hosts (Banerjee, Prakash & Deo, 1970; Soh, 1958).

The chronicity of *A. ceylanicum* infection in hamsters is well illustrated by both egg count and worm burden data. Egg counts remained stable up to 60 days p.i. and small numbers of eggs were detectable for as long as 161 days p.i. This compares favourably with the data of Carroll & Grove (1984) in which a peak egg production of 8000 e.p.g. was reported, 4 weeks p.i. in dogs and was followed by a continuous decline over the following 32 weeks. Worm burdens in our system remained stable for at least 49–56 days whereas Visen *et al.* (1984) observed a gradual reduction in worm burdens from day 18 onwards. This inconsistency between our respective results may be attributable to differences in the strains of hamster which were employed.

Throughout our experiments worms were also sexed and no significant fluctuations in the sex ratios were observed, despite considerable bias in favour of one or other sex in individual hamsters. Out data on the growth and moulting of worms are in broad agreement with the findings of Ray *et al.* (1972): we concluded that worms moulted from L_3 to L_4 on days 3–4 and that the moult to pre-adult worms occurred on days 9–11 (cf. days 2–3 and 6–7 respectively; Ray *et al.* (1972).

Like other hookworms *A. ceylanicum* causes profound blood loss; Areekul, Saenghirum & Ukoskit (1975) estimated the blood loss caused by *A. ceylanicum* in dogs to be 0·0345 ml/worm/day. No comparable data are available for *A. ceylanicum* in hamsters but our measurements of PCV and the haemoglobin data of Menon & Bhopale (1985) indicate significant loss of blood. Weekly monitoring of PCV showed blood loss starting approximately 2 weeks p.i. (Fig. 3), coinciding with the moult to the pre-adult stage. In animals with an estimated burden of 10–30 worms (Fig. 3B) the mean PCV continued to decline until 4 weeks p.i., at which point it stabilized at a value of approximately 40–45%. In animals with higher worm burdens (e.g. mean worm recovery of 30+) we found that PCV continued to decline to 10–20%, a point at which animals were killed to prevent fatal trauma.

In addition to reduced PCV the hamsters also stopped growing and lost weight throughout the initial stages of infection in two of the experiments which were reported (Fig. 4.) In the third experiment 10 infected and 10 control hamsters were housed individually and there was no significant loss of

weight, whereas animals caged in groups as part of the same experiment (data for these groups is not shown) displayed a trend of declining body weight as in Exps 1 and 2. The significant reduction in weight observed in our first two experiments contrasts with the data reported by Menon & Bhopale (1985). It may be significant that DSN hamsters do not grow as large as the strain used by Indian workers, but several other explanations are possible and the reasons for this observation are currently under investigation. There was no disturbance of parasite feeding in individually caged animals as they displayed comparable anaemia to that recorded in other experiments (Fig. 3C).

The anaemia, weight loss, retardation of growth and chronicity of infection seen in the *A. ceylanicum*/DSN hamster model all bear similarities to human ancylostomiasis. The model is more easily manipulable than the dog system or infections in man and it is hoped that our experiments will compliment previous work on hookworms in hamsters, thus providing a firm foundation for future work. The model offers scope for analysing the involvement of acquired immunity and parasite evasive strategies in determining the outcome of infection and should provide a valuable system for screening parasite antigens for incorporation into vaccines.

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