Cross-reactions between *Eimeria falciformis* and *Eimeria pragensis* in mice induced by trickle infections

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SUMMARY

We describe a laboratory model using *Eimeria falciformis* and *E. pragensis* to investigate some of the interactions in double-species infections of *Eimeria*. Mice were given trickle infections by oral inoculation of 100 sporulated oocysts of one species at 3 or 4 day intervals throughout the experiments and, once immunity had developed, as indicated by cessation of oocyst production, the animals were challenged with a single inoculation of the other species. A trickle infection of *E. falciformis* gave a significant enhancement of oocyst output from *E. pragensis* infection as compared with animals that had not received *E. falciformis*. Histological examination of the mice infected with *E. pragensis* showed significantly more asexual parasites and a significantly higher female/male ratio in the mice that had received trickle *E. falciformis* infections than in those that had not. There was no evidence to suggest that extra asexual generations were occurring in these mice. In the converse experimental protocol, trickle *E. pragensis* infections had no significant effect on a single *E. falciformis* infection. We discuss the possible mechanisms for the interactions and also how these interactions may influence multiple species infections in animals in their natural habitats.

Key words: *Eimeria pragensis*, *Eimeria falciformis*, trickle infections, cross-immunity, species interactions.

INTRODUCTION

Eimeria is transmitted from host to host by oocysts which frequently survive for several months in the environment (Fayer & Reid, 1982). If oocysts are present in a susceptible population of wild hosts or hosts farmed under free-range conditions, oocysts are likely to be cycled through the hosts and to be available to infect/reinfect the hosts over long periods; in effect to give trickle infections. The individual doses of oocysts ingested by a host will vary both in size (from 1 oocyst upwards) and in frequency of acquisition. However, even small doses of oocysts (1–100 oocysts) given once or several times are known to induce immunity (Rose, 1974; Joyner & Norton, 1976; Higgs & Nowell, 1988).

Many host species are susceptible to more than 1 species of *Eimeria* (e.g. Levine, 1982; Higgs & Nowell, 1991; Tattersall, Nowell & Smith, 1994) and therefore natural infections, as well as being trickle infections may be multi-specific. In the literature, there are few reports of experimental studies on mixed infections. In some mixed infections, where the 2 parasites occupy the same zone of the gut, competition has been reported, whereas if the parasites occur in separate regions, the combined effect is enhanced (Joyner & Norton, 1983). Enhanced outputs have been reported with multi-specific coccidial infections in lambs (Catchpole, Norton & Joyner, 1976) and in goats (Yvoré, Esnault & Naciri, 1985). The aim of the work presented in this paper is to begin to understand the mechanisms involved. Clearly many combinations of multispecies infections are possible and a simple system was chosen – the effects of a trickle infection of 1 species on a single infection of the second species, the latter infection being given when there was evidence that the trickle infection had induced immunity (shown by lowering of oocyst output). *E. falciformis* and *E. pragensis*, naturally occurring parasites of the house mice (*Mus domesticus*) (Tattersall *et al.* 1994), were employed because their endogenous stages exist in close proximity in the large intestine and therefore any differences of response may be due to the parasite species themselves rather than due to differences of location within the host. We employed individual doses of 100 sporulated oocysts per mouse, an arbitrarily-chosen dose, but possibly approximating to the natural dose that might be picked up by small mammals in the field, although so far as we are aware this dose has never been estimated.

MATERIALS AND METHODS

Experimental animals

Laboratory mice (CD/1 and BALB/c, all males) were obtained from Charles River Ltd (Kent) at 4 weeks of age and were housed in solid-bottom plastic

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cages with wood shavings as bedding, and with PCD diet (Special Diet Services Ltd) and water both provided *ad libitum*. Mice were maintained at 22–25 °C under a 12 h light/12 h darkness regime. Before experimental use, all mice were checked for contamination with *Eimeria* by salt-flotation of faeces. Experimental mice were housed individually in cages in an isolator system (Rawlinson, Nowell & Conisbee, 1985) in a separate room where ambient conditions were the same as those mentioned above. The isolator system and cages were cleaned and then sterilized with gaseous ammonia before each experiment.

Parasites

Both *E. pragensis* and *E. falciformis* were isolated as single oocyst lines from faecal material of a mouse trapped at Moorcopse pig farm (NGR SU729674) by Dr F. Tattersall. The species were characterized by reference to previous descriptions (Cordero del Campillo, 1959; Cerná & Sénaud, 1969; Haberkorn, 1970; Pellérdy, 1974; Owen, 1975). Species were separately maintained, by passage in previously coccidia-free laboratory mice, with oocysts stored in aqueous potassium dichromate solution (2·5 % w/v) in a refrigerator (5 °C), and were extracted from faeces, handled and counted by methods essentially similar to those previously described (Higgs & Nowell, 1988; Nowell & Higgs, 1989). At each infection, mice were inoculated orally with 0·1 ml of a suspension of oocysts (estimated as 1000 sporulated oocysts in 1 ml) of the appropriate species, using a needle (0·9 mm diameter) tipped with a ball of Araldite (about 2 mm diameter) introduced through the mouth into the oesophagus. Oocysts were measured at ×1000 magnification using a calibrated eye-piece graticule where 1 eye-piece unit was equivalent to 1·1 μm.

Histology

Portions (2 cm long) of the caecum, colon or small intestine were removed and immediately fixed in 10 % buffered neutral formalin for not less than 1 day. Intestine was sectioned transversely at 4 μm, and sections were stained in Ehrlich's haematoxylin and eosin. Identification and quantification of the endogenous stages were made by counting parasites in fold crypt units (FCU) defined as a crypt and the epithelium extending up from the crypt to the tip of the folds immediately on either side. Parasites were counted in FCUs on transverse sections, chosen using random number tables.

Experimental methods

In all experiments, mice were randomly allocated to treatments. Inoculations were always timed at 12.00 h ± 2 h and daily collections of total faeces from each mouse were made at 10.00 h ± 2 h. Faecal collection continued at least until the end of quantifiable oocyst output. In all experiments, at least 2 non-inoculated sentinel mice were maintained to check for accidental infection. At the end of each trickle infection the viability of the oocysts was checked by inoculation of oocysts of the same batch into 2 previously infected mice and monitoring the infection. Experimental results are presented such that the day of first inoculation of parasites is day 0. Oocyst counts are given as mean ± standard error of the mean.

For each species of *Eimeria*, oocyst outputs resulting from single inoculations were used to provide baseline data for subsequent studies on trickle infections and the challenge with the other species.

Trickle infections of 1 species were given by inoculating animals up to 12 times at 3 or 4 day intervals. The challenge inoculation of the other species was made when the oocyst output from the trickle infection was no longer detectable using the McMaster slide technique. In each experiment there were 3 groups of mice: Group A, given the trickle infection alone, Group B, given the trickle infection followed by the challenge infection, and Group C, given the challenge infection alone.

To check that it was not the act of inoculation that might make animals more susceptible to challenge, an experiment was performed where a placebo trickle infection was given (0·1 ml deionized water) to a group of mice which were compared with untreated mice in their response to a challenge inoculation of *E. pragensis*.

RESULTS

The sentinel mice did not show oocysts in their faeces in any of the experiments. As judged by their ability to induce oocyst production in test mice inoculated at the end of each experiment, suspensions of oocysts used during trickle infections remained viable throughout each experiment.

Single infections of E. falciformis *and* E. pragensis

In both CD/1 and BALB/c mice, a single inoculation of *E. falciformis* produced an oocyst output which started on day 5 post-inoculation (p.i.). Patency, with oocyst levels that could be estimated, continued until day 8 p.i. although a few oocysts were produced after this time. Over 95 % of oocyst output occurred within the first 3 days of patency (days 5–7 p.i.) although the total output and the day of peak output were variable (Table 1, Fig. 1).

With a single inoculation of *E. pragensis*, there was again variability, but mice of both strains started to shed oocysts on day 8 p.i., with patency usually lasting until day 12 and with over 95 % of oocyst production being on days 8–11 p.i. (Table 1, Fig. 1).

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Table 1. *Eimeria falciformis* and *E. pragensis*: characteristics of infections resulting from single inoculations in mice

(Each mouse was inoculated with an estimated dose of 100 sporulated oocysts.)

Group	Mice (*n*)	Peak days	Total oocyst output (mean ± S.E.)
E. falciformis			
BALB/c (batch 1)	6	6, 6, 5, 5, 5, 5	$6{\cdot}74\times10^{6}\pm0{\cdot}48\times10^{6}$
CD/1 (batch 1)	7	6, 6, 6, 6, 6, 6, 5	$8{\cdot}83\times10^{6}\pm1{\cdot}52\times10^{6}$
CD/1 (batch 2)	6	7, 7, 6, 6, 5, 5	$6{\cdot}94\times10^{6}\pm0{\cdot}65\times10^{6}$
E. pragensis			
BALB/c (batch 2)	8	9, 9, 9, 9, 8, 8, 8, 8	$6{\cdot}98\times10^{6}\pm0{\cdot}51\times10^{6}$
CD/1 (batch 3)	6	10, 9, 9, 9, 9, 8	$7{\cdot}43\times10^{6}\pm0{\cdot}79\times10^{6}$
CD/1 (batch 4)	8	9, 9, 9, 8, 8, 8, 8, 8	$2{\cdot}80\times10^{6}\pm0{\cdot}22\times10^{6}$

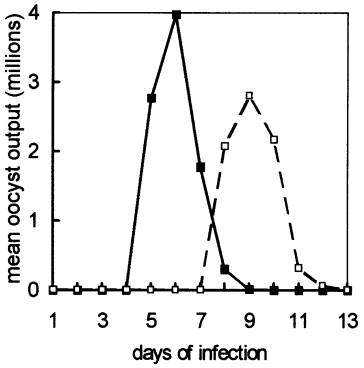

Fig. 1. Mean oocyst output curves for single infections of *Eimeria falciformis* (——) or of *E. pragensis* (– – – –) in CD/1 mice. Mean ± S.E. ($\times10^{-6}$) on peak day are $3{\cdot}98\pm0{\cdot}61$ and $2{\cdot}80\pm0{\cdot}39$ respectively. Infections were given on day 0. Dose estimated as 100 sporulated oocysts/mouse. For each infection, $n = 6$.

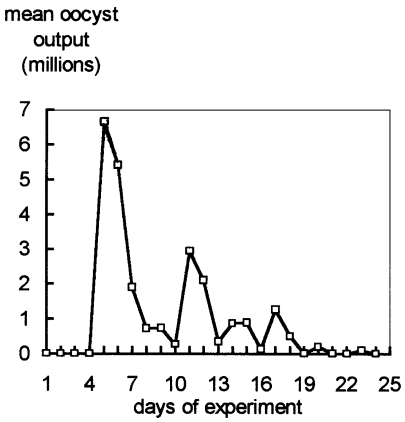

Fig. 2. Mean oocyst output curve for CD/1 mice given trickle infections (estimated as 100 sporulated oocysts/dose) of *Eimeria falciformis* on days 0, 3, 6, 9, 12, 15 and 18 of the experiment; $n = 8$. Mean ± S.E. ($\times10^{-6}$) total oocyst output/mouse is $25{\cdot}14\pm1{\cdot}93$.

Trickle infection of E. falciformis *and challenge with single infection of* E. pragensis (*Protocol 1*)

Both CD/1 and BALB/c mice given trickle infections of *E. falciformis* showed series of peaks of oocyst output which, with allowance for the period of endogenous development, correlated with the times of inoculation (Fig. 2). The trend was for output to reduce, finally to levels below detection.

In an experiment where CD/1 mice were trickle infected (12 doses between days 0 and 39) and then challenged with *E. pragensis* (day 26), mice produced detectable numbers of oocysts of *E. falciformis* up to day 24 of the experiment. After inoculation with *E. pragensis*, mice of Groups B and C produced similarly timed output curves of oocysts typical of that species, starting on day 34, i.e. day 8 after inoculation of *E. pragensis* (day 8 p.i. *E.p.*). For Group B, the total output (days 8–11 p.i. *E.p.*) was significantly higher (*t*-test: $t = 3{\cdot}4898$, D.F. = 10, $P < 0{\cdot}01$) (Table 2, Fig. 3). The mean output on the day of peak oocyst production (day 9 p.i. *E.p.*) was also significantly higher for Group B (*t*-test: $t = 3{\cdot}1020$, D.F. = 10, $0{\cdot}02 > P > 0{\cdot}01$). Mice of Group A, not inoculated with *E. pragensis*, did not show a peak of oocyst production at this time.

Measurements of oocyst widths taken from single mice of Group B and of Group C during the peak days of *E. pragensis* infection were similar (B $18{\cdot}4\pm0{\cdot}02\ \mu$m; C $18{\cdot}6\pm0{\cdot}06\ \mu$m, $n = 300$ in each case), but differed significantly from the widths of *E. falciformis* oocysts derived from mice inoculated with this species as a viability check ($14{\cdot}3\pm0{\cdot}05\ \mu$m, $n = 300$) (1-way ANOVA, $F = 2873{\cdot}98$, $P = 0{\cdot}001$). With the measurement classes used, there was some overlap of width between the species, about 1·3% and about 7% of *E. falciformis* and *E. pragensis* respectively, falling within the 16·5–17·5 μm class (Fig. 4A, C).

BALB/c mice showed similar patterns to those in CD/1 mice, for both the *E. falciformis* trickle infection (11 doses, days 0–35) and the subsequent *E. pragensis* single infection after inoculation on day 26 (Table 2). The total oocyst output from mice inoculated with *E. pragensis* was higher for Group B than for Group C although not significantly so (*t*-test: $t = 1{\cdot}7595$, D.F. = 14, $P > 0{\cdot}1$). However, the output on day 9 p.i. *E.p.*, the mean peak day for the

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Table 2. *Eimeria falciformis* and *E. pragensis*: effects of a trickle infection on a subsequent single infection of the heterologous species

(The table summarizes oocyst output data of the single infections from 3 experiments detailed in the text. Mean ± S.E. and significant (S) or non-significant (N.S.) differences at 5% level are stated.)

Single infection		Mouse pre-treatment	
E. pragensis	Oocyst output	*E. falciformis* trickle infection	No prior infection
CD/1 ($n = 6$)	Total:	15·38 ± 2·20	7·05 ± 0·78 s
	On day 9:	6·99 ± 1·29	2·80 ± 0·39 s
BALB/c ($n = 8$)	Total:	7·80 ± 0·51	6·85 ± 0·50 N.S.
	On day 9:	5·93 ± 0·45	3·16 ± 0·50 s
E. falciformis	Oocyst output	*E. pragensis* trickle infection	No prior infection
CD/1 ($n = 6$)	Total:	5·67 ± 0·54	6·43 ± 0·36 N.S.
	On day 6:	3·15 ± 0·20	2·60 ± 0·25 N.S.

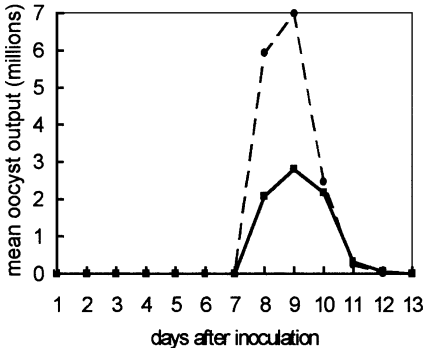

Fig. 3. Mean oocyst output curves for single infections (estimated as 100 sporulated oocysts/mouse) of *Eimeria pragensis* in CD/1 mice. Infections were given on day 0. Mice previously given a trickle infection of *E. falciformis* (----); mice previously uninfected with coccidia (——). See Table 2 for details of variance. For each curve, $n = 6$.

group, was significantly higher (*t*-test: $t = 4·1182$, D.F. = 14, $P < 0·01$). Oocysts from mice of Groups B and C taken on day 9 p.i. *E.p.* did not differ significantly in width (for each group, $n = 800$; *t*-test $t = -0·65$, $P = 0·52$) and were typical of *E. pragensis*.

Trickle infection of E. pragensis *and challenge with single infection of* E. falciformis (*Protocol 2*)

Trickle infections of *E. pragensis* in CD/1 mice followed the same pattern as *E. falciformis*, but with the longer period of endogenous development, the peaks of oocyst output occurred approximately 8–10 days after each inoculation (Fig. 5).

In an experiment where CD/1 mice were trickle infected with *E. pragensis* (11 doses, days 0–35) and then challenged with *E. falciformis* (day 26), mice produced detectable numbers of oocysts of *E. pragensis*, up to day 21 of the experiment. One mouse also produced a measurable output on day 30. After inoculation with *E. falciformis*, mice of Groups B and C produced similarly timed oocyst output curves starting on day 31 (day 5 p.i. *E.f.*) typical of *E. falciformis*, but neither the total oocyst output figure nor the output on the mean peak day (day 6 p.i. *E.f.*) for the 2 groups were significantly different (*t*-test D.F. = 10, $t = 1·007$, $P > 0·1$ and D.F. = 10, $t = 1·5868$, $P > 0·1$ respectively) (Table 2).

The variation of width of sporulated oocysts from Group B mice after *E. falciformis* infection is shown in Fig. 4B. This oocyst width distribution differed from that after a single *E. falciformis* inoculation (Fig. 4A) in the number of oocysts $\geqslant 16·5\ \mu$m (18·5% and 1·3% respectively).

E. pragensis *infection after a placebo trickle infection*

Mice which were orally inoculated with water (equivalent to a trickle infection with 11 doses each of zero oocysts, days 0–35) and mice not so treated, were inoculated with *E. pragensis* on day 28. The total output from the treated mice was higher than from those that were untreated ($2·90 \times 10^6 \pm 0·87 \times 10^6$ and $2·82 \times 10^6 \pm 0·21 \times 10^6$ oocysts respectively) but the difference was not significant (*t*-test $t = 0·2436$, D.F. = 14, $P > 0·1$).

Endogenous stages of E. pragensis *in* E. falciformis-*immune mice*

In mice with single infections of either species, parasites were seen in enterocytes of the caecum and colon, but never in cells of the small intestine. *E. falciformis*-parasitized cells of the superficial epithelium near the intestinal lumen, whilst *E. pragensis* was in the deeper epithelium within the crypts. The endogenous stages closely resembled the descriptions given by Cerná & Sénaud (1965) and Owen (1975).

After trickle infection with *E. falciformis*, whether or not subsequently challenged with *E. pragensis*, a few isolated endogenous stages of *E. falciformis* occurred in the superficial epithelium. After *E. pragensis* challenge of both *E. falciformis*-immune mice and previously uninfected controls, similar endogenous stages were commonly seen in the deep

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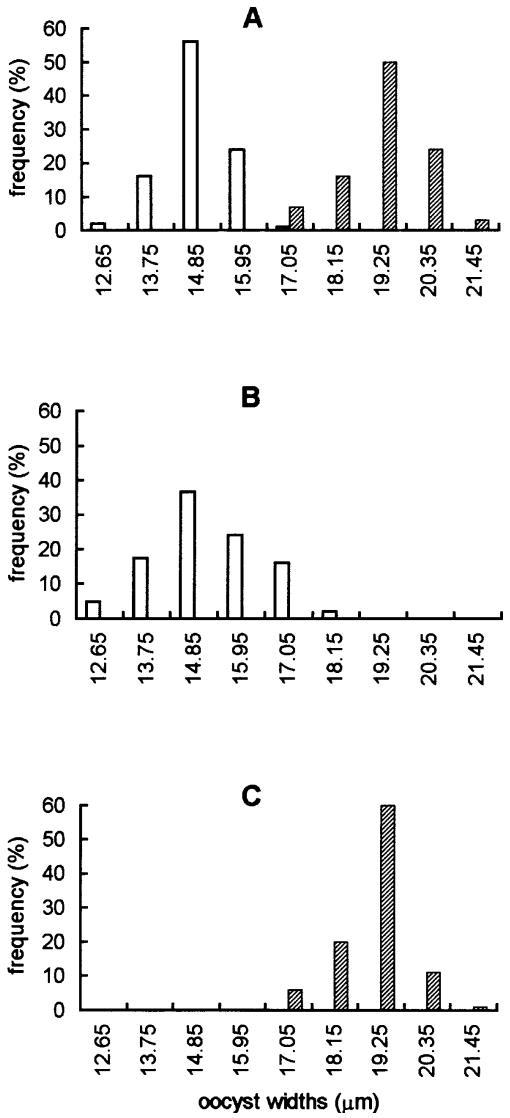

Fig. 4. Percentage frequency histograms of widths of sporulated oocysts of *Eimeria falciformis* (□) and of *E. pragensis* (▨) taken from mice during times of peak input. Mid-points of width classes (1·1 μm) are marked, thus 12·65 indicates the class from 12·1 to 13·1 μm. (A) Oocysts of each species taken from single infections in CD/1 mice. For each species, $n = 300$. (B) Oocysts of *E. falciformis* taken from mice recovered from a trickle infection of *E. pragensis*; $n = 1476$. (C) Oocysts of *E. pragensis* taken from mice recovered from a trickle infection of *E. falciformis*; $n = 1800$.

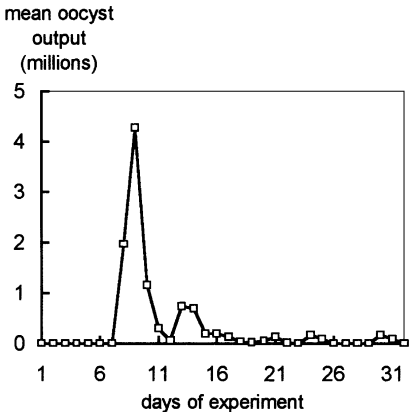

Fig. 5. Mean oocyst output curve for CD/1 mice given trickle infections (estimated as 100 sporulated oocysts/dose) of *Eimeria pragensis* on days 0, 4, 7, 11, 14, 18, 21, 25, 28, 32 and 35 of the experiment; $n = 6$. Mean ± S.E. ($\times 10^{-6}$) total oocyst output/mouse is 10·44 ± 1·22.

epithelium of both sets of mice between days 5 and 8 after infection, and these were like the stages described for single infections. Stages were not seen on days 1–4. Statistical analysis of the total number of stages in the caecum and in the colon (Table 3) shows that significantly more stages occurred in the *E. falciformis*-immune as compared with the control mice on days 5–8 (Minitab balanced ANOVA. caecum: D.F. = 1, $F = 19{\cdot}82$, $P = 0{\cdot}002$ and colon: D.F. = 1, $F = 15{\cdot}93$, $P = 0{\cdot}004$). A comparison of stages in the caecum and in the colon showed no significant difference between the 2 organs for *E. falciformis*-immune mice and for control mice (Minitab 2-sample *t*-test. $T = 0{\cdot}37$, D.F. = 14, $P = 0{\cdot}36$ and $T = 0{\cdot}23$, D.F. = 14, $P = 0{\cdot}41$ respectively).

The ratios of numbers of macrogametocytes to microgametocytes (female/male ratio) calculated separately for caecum and colon on days 6 and 7 after infection with *E. pragensis* in the 2 groups of mice are shown in Table 4. The ratios in the caecum for the 2 groups did not differ significantly (Minitab balanced ANOVA D.F. = 1, $F = 0{\cdot}42$, $P = 0{\cdot}553$), but in the colon this ratio was significantly higher in the *E. falciformis*-immune mice than in the control mice (Minitab balanced ANOVA D.F. = 1, $F = 13{\cdot}5$, $P = 0{\cdot}021$).

DISCUSSION

Although some authors consider *E. falciformis* and *E. pragensis* to be synonymous (Levine & Ivens, 1990), we believe that the 2 parasite lines used in this work were sufficiently different in terms of oocyst dimensions, pre-patent period and sites of endogenous development, to be treated as separate species. These characteristics are in agreement with previously published literature (see above).

Except for differences due to the pre-patent periods, trickle infections of *E. falciformis* and *E. pragensis* progressed similarly. With both species, the mice developed a strong homologous immunity as demonstrated by the reduction and termination of oocyst production: similar to *E. hungaryensis* infections in *Apodemus sylvaticus* (Higgs & Nowell, 1988). After oocyst production had stopped in CD/1 mice exposed to trickle infection with *E. falciformis*, a few endogenous stages were still found in the enterocytes. It is uncertain whether these parasites were derived from a recent inoculum or whether they were derived from an earlier dose, but in a state of suspended development. There were individual variations of

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Table 3. *Eimeria pragensis*: mean number (±S.E.) of life-cycle stages counted in caecum and colon samples of *E. falciformis*-immune and control mice

(n = 50 fold crypt units/day for 2 mice in each group.)

Days p.i.*	Main endogenous stages	*E. falciformis*-immune mice		Control mice	
		Caecum	Colon	Caecum	Colon
5	3° Schizonts	26·0±0·36	21·8±0·35	24·2±0·52	19·5±0·31
6	4° Schizonts/immature sexual stages	24·5±0·75	24·3±0·53	20·0±0·52	22·8±0·45
7	4° Schizonts/immature and mature sexual stages	88·4±1·62	69·3±0·93	56·7±1·13	48·5±0·89
8	Mature sexual stages	50·2±0·60	53·5±1·40	38·8±0·75	42·3±0·97

* Days post-inoculation with *E. pragensis*.

Table 4. *Eimeria pragensis*: ratios of female to male gametocytes in sections of caecum and colon of *E. falciformis*-immune and control mice sampled on each of days 6 and 7 after inoculation with *E. pragensis*

(Four mice/day (2 mice/day/group). n = 20-fold crypt units/mouse.)

Days p.i.*	Mouse	*E. falciformis*-immune mice		Control mice	
		Caecum	Colon	Caecum	Colon
6	1	2·33	5·00	3·33	2·13
6	2	4·17	4·55	1·79	1·67
7	3	4·17	8·33	3·03	4·35
7	4	4·35	3·70	5·56	3·03

* Days post-inoculation with *E. pragensis*.

the oocyst output in CD/1 mice, an outbred line, and to reduce any experimental variation resulting from mouse genotype, we repeated some experiments with BALB/c mice (inbred), but to no obvious advantage.

Although unpublished results from immunohistochemical staining of parasites in tissue sections indicated that there was 2-way cross-reactivity of antibodies, experiments using protocols 1 and 2 gave no evidence of heterologous immunity suppressing the single challenge infection. On the contrary, *E. pragensis* infection was enhanced in mice that had become immune to *E. falciformis* after trickle infection (protocol 1) whilst the reverse protocol of infection (protocol 2) did not produce the same enhancement. Under protocol 1, the pre-patent period and the oocyst width measurements indicate that the oocyst output after challenge may be regarded as being of the second species alone. Although oocyst output numbers increased, lack of change of pre-patent or patent periods suggests that the timing of the endogenous stages during the challenge infection was not affected by the trickle infection. This conclusion, concerning lack of change of pre-patent and patent periods, also holds true for experiments under protocol 2. However, under protocol 2 the output from the challenge infection did contain some larger oocysts, possibly of *E. pragensis* derived from the trickle infection.

The present work shows that in protocol 1, enhancement of *E. pragensis* output involves at least 2 stages: an increase in the number of endogenous stages, certainly on days 5–8 of infection and possibly before then as well; and an increase in the female/male gamont ratio in the colon, if not in the caecum. However, there was no evidence of an increased number of schizogonic generations.

There are several questions that need to be addressed: first, why and how should infection with one species of *Eimeria* enhance another; and second, why and how should this enhancement not occur in all combinations of *Eimeria* species.

A similar enhancement has been noted between some other species of *Eimeria* in hosts other than mice, although there has been no detailed explanation of the mechanisms involved (Duszynski, 1972; Rose, 1975; Yvoré *et al*. 1985). These results (except for Rose, 1975) differ from the present work because in the earlier studies the interactions were in animals before any significant anticoccidial immunity had developed and the inoculations of the 2 or more species of *Eimeria* were not always synchronous although their patencies (times of oocyst output) were. From these previous studies and also from general observations of multiple species infections, 2 possible types of interaction have been suggested: direct interactions between the parasites (Duszynski, 1972; Kheysin, 1972; Hein, 1976; Joyner & Norton, 1983); and indirect interactions, for instance the induction of pathophysiological changes by one species weakening the host response to infection by the other (Duszynski, 1972).

Direct interactions seem to be an unlikely explanation for the enhancement in protocol 1 because, although the trickle infection was still being administered at time of challenge, there was a paucity of *E. falciformis* parasites. As for indirect interactions, there are various possible explanations: changes of lymphocyte and macrophage activity (Huff & Clark,

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1970; Rose, 1974; Rose, Hesketh & Rennie, 1984; Bhogal *et al*. 1986), structural modifications of the parasitized cell (Augustine, 1996; Fernando, 1986) and immunosuppression (Rose & Hesketh, 1984).

Acquired immunity against *Eimeria* is recognized as being very species specific (Kogut, 1990) with the major component of parasite neutralizing immunity being cell-mediated: T-cell dependent with the cytokines being stimulatory to further lymphocytes and macrophages (Wakelin & Rose, 1990; Rose, Hesketh & Wakelin, 1992). These cell types may have a double effect on *Eimeria*: not only are they important in the destruction of the parasite, but they are also believed to enhance the infection for instance by transporting the parasites during the life-cycle (Doran, 1966; Al-Attar & Fernando, 1987; Trout & Lillehoj, 1993; Ovington, Alleva & Kerr, 1995). Since the cytokines are produced by a specific immune response but are non-specific in their effects, it is suggested that *E. falciformis* may have stimulated proliferation of lymphocytes and macrophages, and some of these cells subsequently aided the life-cycle stages of *E. pragensis*. The phenomenon of cytokine enhancement is believed to occur in infections with other pathogens (Amiri *et al*. 1992; Kurane & Ennis, 1992).

Cell-culture work by Augustine (1996) suggests that besides immune responses there may be at least a transitory effect of infection by one species on the host cells making them more susceptible to invasion by other species, but it is not clear how far this effect may spread between cells. The enterocytes parasitized by *E. falciformis* are at the tips of the folds whilst those inhabited by *E. pragensis* are deeper between the folds and hence their habitats are not identical. Also, since cell migration within the folds is from crypt to tip (Fawcett, 1986), it is unlikely that by their position, *E. falciformis*-parasitized cells would become susceptible to attack by *E. pragensis*.

Suppression of immunity is known to be induced by several parasitic infections including *Eimeria* (Rose & Hesketh, 1984; Rose, Wakelin & Hesketh, 1994). Also experimental immunosuppression of mice leads to production of elevated levels of *E. pragensis* oocysts, possibly because of increased numbers of asexual stages (Rose & Hesketh, 1986). In the present experiments, one source of immunosuppression might be the stress resulting from the act of repeated inoculation of oocysts for the trickle infection. However, the experiments involving trickle inoculation with water (zero oocysts) and also those using protocol 2 did not suggest that this type of stress was important.

It is unclear why protocol 2 did not produce the same effect as protocol 1. Again, direct and indirect effects produced by *E. pragensis* would be expected to influence *E. falciformis*. It may be that either the effects induced by *E. pragensis* were weaker than those of *E. falciformis*, or alternatively there were enhancing effects but these were neutralized by some unknown antagonistic effects not seen in protocol 1. Certainly from the argument (see above) about the positioning of the endogenous stages and host-cell migration, a relevant point may be that prior *E. pragensis* infection could make the cells appropriate for *E. falciformis* infection less suitable for the latter parasite.

Some characteristics of naturally occurring *Eimeria* infections may be explained by the observations of the present paper. Many species of mammals including rodents are recorded as being hosts to several species of *Eimeria* (Ball & Lewis, 1984; Levine & Ivens, 1990; Lindsay & Todd, 1993; Tattersall *et al*. 1994). In populations of wild rodents, prevalences of at least 35% have been recorded, with 2 or more species sometimes being present as concurrent infections (e.g. Ball & Lewis, 1984; Nowell & Higgs, 1989; Fuller, Hefner & Wrosch, 1995). Since *Eimeria* spp. are highly host-specific, and if solid immunity to each species does develop, one might expect that the adult animals of a susceptible host population would become free of *Eimeria* infection. In practice this is not the case, Ball & Lewis (1984) having shown that in older rodents (*Myocaster coypus* and *Apodemus sylvaticus*), the prevalence rates are reduced significantly over those in juvenile animals, but remain above 20%. The enhancing interactions, at least between some species of *Eimeria* and in some circumstances, may be one way in which prevalence rates remain high through the various age classes of a host population.

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