Schistosoma japonicum and *Trichuris suis* infections in pigs fed diets with high and low protein

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SUMMARY

The aim of the study was to measure the impact of *Schistosoma japonicum* and *Trichuris suis* infections in young growing pigs fed low- or high-protein diets. Thirty-two pigs, 6–10 weeks old, were randomly allocated to 2 groups receiving either a high- or a low-protein diet. After 11 weeks half of the pigs from each group were infected with 1500 *S. japonicum* cercariae and 4000 *T. suis* eggs. The weight of the pigs was measured throughout the study, and blood and faecal samples were collected every second week from the time of infection. At the time of infection the low-protein pigs had significantly lower mean body weights, haemoglobin and albumin levels compared with the high-protein pigs, and this pattern continued throughout the study. The serum albumin concentration was further significantly reduced in the infected low-protein pigs compared to the non-infected low-protein pigs. Significantly more *S. japonicum* worms as well as faecal and tissue eggs were found in the low-protein pigs compared with the high-protein pigs. No differences between the 2 diet groups were observed in *T. suis* establishment rates or faecal egg excretion. We conclude that this low-protein diet increased the establishment rates of *S. japonicum*, favoured larger deposits of *S. japonicum* eggs in the liver and faecal egg excretion, reduced weight gains and caused anaemia and hypoalbuminaemia in young growing pigs as compared with a high-protein diet.

Key words: *Schistosoma japonicum*, *Trichuris suis*, pig, low-protein diet.

INTRODUCTION

The potential impact of *Schistosoma japonicum* and *Trichuris trichiura* infections on nutritional status and morbidity in humans has been described in human studies (Gilman *et al*. 1983; Stephenson, 1987; Bundy & Cooper, 1989; McGarvey, 1992; Simeon *et al*. 1994; McGarvey *et al*. 1996; Wiest, 1996). The majority of these studies have focused on the relationship between parasitism and nutrition, health and cognition as well as the improvements achieved by removal of the parasites with anthelmintic drug treatment.

Experimental infections in humans of different nutritional status are not possible for ethical reasons. However, animal model systems employing closely related host and parasite species may help determine the complex interaction between helminth infections and nutritional status of the host. Recently, the pig–*S. japonicum* model was proposed for studying human schistosomiasis japonica (Willingham & Hurst, 1996). Anatomical, physiological, immunological, physical and other characteristics of the pig are very similar to those in humans, and only surpassed by non-human primates (Stephenson, 1987). Furthermore, the pig is a natural final host of the Chinese blood fluke, *S. japonicum*; and *T. suis*, also a natural parasite of the pig, is very closely related to *T. trichiura* in humans.

No controlled studies have been done using experimental infections with both *S. japonicum* and *T. suis* in groups of pigs with different nutritional status. We report here an experimental study of the relationship between low and high protein diets, weight gain, haemoglobin and albumin levels and the establishment and fecundity of simultaneous infections with the trematode, *S. japonicum* and the nematode, *T. suis*, in growing pigs.

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MATERIALS AND METHODS

Experimental design

Thirty-two specific pathogen-free Danish Landrace/Yorkshire/Duroc cross-bred female pigs were housed together randomly under helminth-free conditions after weaning at an age of 4 weeks. The pigs were divided into 2 groups of 16 pigs to which either a high- or low-protein diet was offered *ad*

Table 1. Chemical composition of the two experimental diets

	High-protein diet	Low-protein diet
Dry matter (%)	91·6	90·9
Ash (%)	6·2	4·9
Protein (%)	24·6	13·8
Fat (%)	8·7	5·4
Carbohydrate + lignine (%)	60·5	75·9
Lysine (g/kg)	15·5	8·4
Methionine (g/kg)	4·7	2·6
Cysteine (g/kg)	3·4	1·9
Threonine (g/kg)	8·7	4·8
Fe (mg/kg)	383	339
Zn (mg/kg)	262	247
Cu (mg/kg)	199	196

libitum (Table 1). The protein content in the low-protein diet was approximately half (13·8% of the dry matter) of that in the high-protein diet (24·6% of dry matter). So, more carbohydrate was added to the low-protein diet to achieve equal feed units (1·36/kg). This, however, resulted in a slightly higher digestibility in the low-protein diet (94·5 and 91·3% of dry matter, respectively). The concentration of the 4 essential amino acids, lysine, methionine, cysteine and threonine was halved in the low-protein diet. The concentrations of vitamins and minerals in the two diets were similar.

After 11 weeks on the experimental diets, 8 pigs in the low-protein group and 8 pigs in the high-protein group were each infected with both 1500 *S. japonicum* cercariae and 4000 infective *T. suis* eggs. The *S. japonicum* isolate originated from Anhui Province, People's Republic of China, and is maintained in *Oncomelania hupensis hupensis* at the Danish Bilharziasis Laboratory. The cercariae were administered using the intramuscular route of infection according to the method described by Willingham *et al*. (1996). The infective eggs of *T. suis* were originally isolated from soil from a small organic farm and subsequently passaged once in helminth-naïve pigs. The eggs obtained from the faeces of these pigs were embryonated according to the method described by Burden & Hammet (1976) and were administered orally via a stomach tube. All pigs were weighed approximately every 2 weeks during the experimental period.

Parasitology and haematology

Adult and immature worms of *S. japonicum* were recovered from the pigs according to the method described by Willingham *et al*. (1997) and their sex and number per pig determined. Pigs were sedated by an intramuscular injection of 0·06 ml/kg of Zoletil (zolazepam/tiletamin) and 0·1 mg/kg of Narcoxyl (Xylazinum NFN). Adult *T. suis* worms were recovered according to a method described by Roepstorff & Murrell (1997). Faecal samples were collected from the rectum of each pig every second week from the time of infection, and *S. japonicum* faecal egg counts were determined according to the method described by Willingham *et al*. (1997). *T. suis* egg counts were performed using the McMaster egg count technique. Enumeration of *S. japonicum* eggs in the liver was performed as described by Bøgh *et al*. (1996).

All pigs were bled biweekly from the time of infection and throughout the study; haemoglobin concentrations (mMol/l) and serum albumin levels (g/l) were measured using a Coulter Counter and eosinophil counts were determined by conventional counting after staining with eosin.

Statistical analyses

Student's *t*-test was used to estimate the probability of significant differences between the two infected groups in mean number of *S. japonicum* and *T. suis* worms, *S. japonicum* tissue egg counts and *S. japonicum* and *T. suis* faecal egg counts. Faecal egg counts were $\log 10\ (x+1)$ transformed before the analyses. Student's *t*-test was also applied to assess the differences in mean body weight, eosinophil counts, haemoglobin and albumin levels before infection with *S. japonicum* and *T. suis*. One-way analysis of variance was used to compare group means of body weight, eosinophil counts, haemoglobin and albumin levels following infection. Repeated measures analysis of variance was applied to determine the impact of either low-protein diet or parasite infections on body weight, eosinophil counts, haemoglobin and albumin levels. Adjustment for the effect of differences in body weight on faecal egg excretion and fecundity was made according to digestibility and dry matter contents in faeces.

RESULTS

During the study 2 pigs (1 from the infected high-protein group and 1 from non-infected high-protein group) developed chronic arthritis and were therefore excluded from the study. Apart from light diarrhoea 6–8 weeks post-infection, no clinical signs related to either *S. japonicum* or *T. suis* infections were noted at any time during the study period.

Body weight

The mean body weights of the pigs in the 4 different groups are shown in Fig. 1. The pigs were followed from 11 weeks before the parasite infections were given until 11 weeks post-infection. From 2 weeks

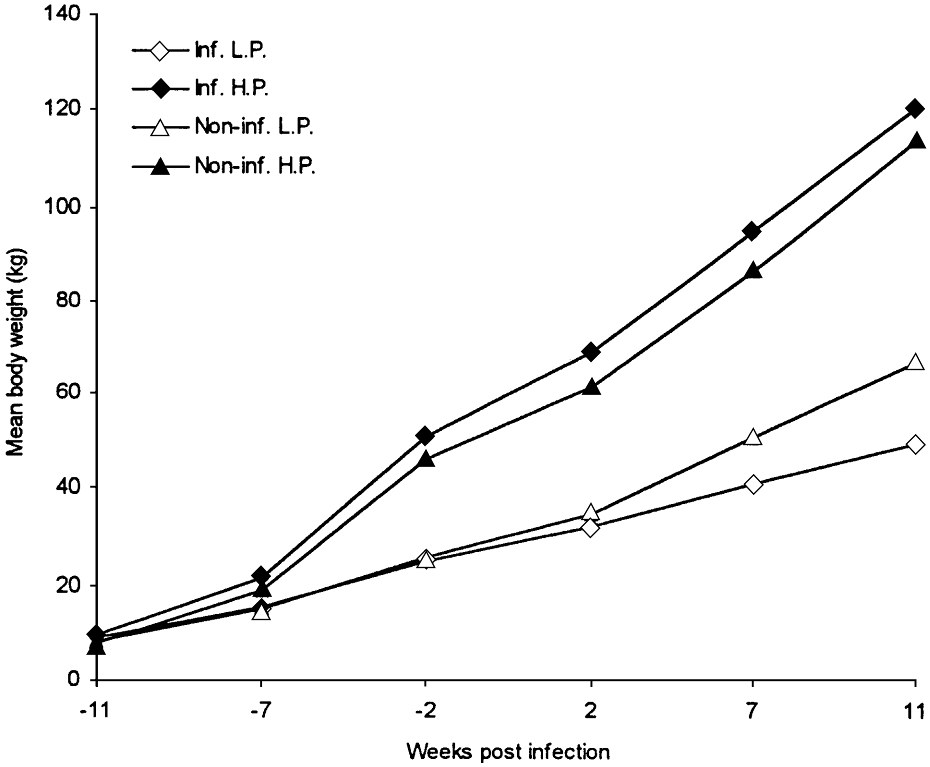

Fig. 1. Mean body weight of pigs fed either a low- or a high-protein diet and infected with *Schistosoma japonicum* and *Trichuris suis*. Inf. L.P.: infected low-protein group; Inf. H.P.: infected high-protein group; Non-inf. L.P.: non-infected low-protein group; Non-inf. H.P.: non-infected high-protein group. Significant group differences were seen from 2 weeks before infection: Inf. L.P. and Non-inf. L.P. < Inf. H.P. and Non-inf H.P. ($P < 0{\cdot}01$).

Table 2. Mean number ± s.d. of *Schistosoma japonicum* and *Trichuris suis* worms and establishment rates as well as liver egg counts for *S. japonicum*

	Mean number of worms and liver eggs/g ± s.d.		
	Low-protein group (8 pigs)	High-protein group (7 pigs)	Student's *t*-test *P* value
S. japonicum			
Males	18·4 ± 11·3	2·1 ± 1·3	$P < 0{\cdot}01$
Females	17·0 ± 10·2	2·4 ± 2·0	$P < 0{\cdot}01$
Immatures	1·0 ± 1·2	0·6 ± 1·1	n.s.
Total	36·4 ± 21·8	5·1 ± 3·7	$P < 0{\cdot}01$
Establishment (%)	2·4 ± 1·4	0·3 ± 0·2	$P < 0{\cdot}01$
Liver egg counts/g	485·6 ± 483·2	36·0 ± 35·3	$P < 0{\cdot}05$
T. suis			
Total	1784·4 ± 940·7	1354·3 ± 1699·2	n.s.
Establishment (%)	44·6 ± 23·5	33·9 ± 42·5	n.s.

n.s., No significant difference.

before infection and for the rest of the study period the mean body weights in the 2 low-protein groups were significantly lower than in the high-protein groups ($P < 0{\cdot}01$). Although the mean body weight increase was reduced in the infected low-protein group at the last weighing time as compared with the non-infected low-protein group this was not statistically significant. With analyses of repeated measurements the difference in body weight between the groups could be explained by the different diets given and not by the different parasitological status of the groups ($P < 0{\cdot}001$).

Worm recovery and tissue egg counts

The number of *S. japonicum* and *T. suis* worms recovered, the establishment rates, and *S. japonicum* liver egg counts are presented in Table 2. The total

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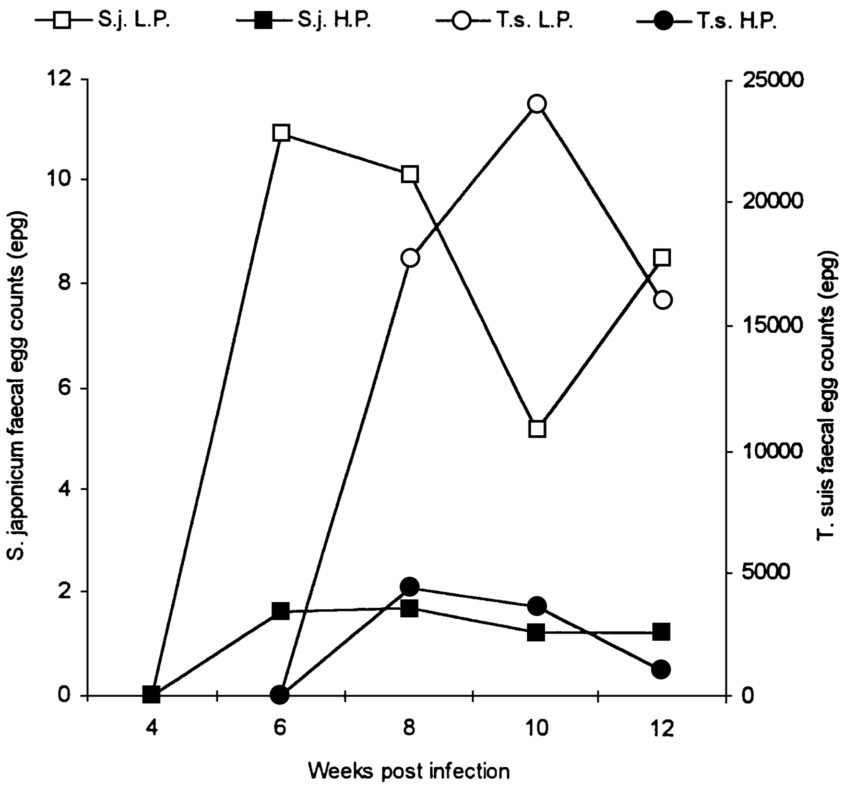

Fig. 2. Geometric mean faecal egg counts of *Schistosoma japonicum* and *Trichuris suis*-infected pigs. S.j. L.P.: *S. japonicum* mean faecal egg counts in low-protein group; S.j. H.P.: *S. japonicum* mean faecal egg counts in high-protein group; T.s. L.P.: *T. suis* mean faecal egg counts in low-protein group; T.s. H.P.: *T. suis* mean faecal egg counts in high-protein group. Significant group differences were seen between the *T. suis*-infected groups 8, 10 and 12 weeks post-infection: T.s. L.P. < T.s. H.P. ($P < 0{\cdot}05$).

number of *S. japonicum* worms recovered and the worm establishment rates were significantly higher in the infected low-protein group ($P < 0{\cdot}01$) as compared with the infected high-protein group. The *S. japonicum* liver egg counts were also significantly higher in the low-protein group compared with the high-protein group ($P < 0{\cdot}05$). *T. suis* worm establishment did not significantly differ between the 2 infected groups.

Faecal egg counts

The administration of *S. japonicum* cercariae and *T. suis* larvae resulted in faecal egg excretion of both parasites in all infected pigs.

The geometric mean faecal egg counts for both *S. japonicum* and *T. suis* are presented in Fig. 2. Comparison of schistosome faecal egg counts revealed a statistically significant higher egg output in the infected low-protein group compared with the infected high-protein group from week 6 through to the end of the study ($P < 0{\cdot}05$). In contrast, the number of *T. suis* eggs excreted in faeces was only significantly higher 12 weeks post-infection in the low-protein group compared with the high-protein group ($P < 0{\cdot}05$). However, when the data were adjusted for the estimated amount of faeces excreted/day/pig, *S. japonicum* total egg excretion/day was found to be significantly higher in the low-protein group only at weeks 6 and 12 post-infection ($P < 0{\cdot}05$), and for *T. suis* no significant difference was observed at any time. Comparison of the mean number of *S. japonicum* faecal eggs/female worm excreted at the last sampling time between the 2 infected groups revealed a significantly higher fecundity in the low-protein group ($0{\cdot}9 \pm 0{\cdot}9$) as compared with the high-protein group ($0{\cdot}1 \pm 0{\cdot}3$; $P < 0{\cdot}05$). However, no significant difference in worm fecundity was observed after adjusting for the estimated difference in total faecal matter excreted/day between the 2 groups. For *T. suis* no significant difference in worm fecundity was observed either before or after adjustment for estimated total daily faecal excretion.

Blood parameters

At the time of infection the mean haemoglobin level in the low-protein group was significantly lower ($P < 0{\cdot}001$) than the level in the high-protein group (Fig. 3). Two weeks after infection no significant difference was seen between any of the groups but from 4 weeks post-infection the haemoglobin levels remained at a significantly lower level in the low-protein groups compared with the high-protein groups independent of parasite status ($P < 0{\cdot}05$). Only the diet was found to be associated with the

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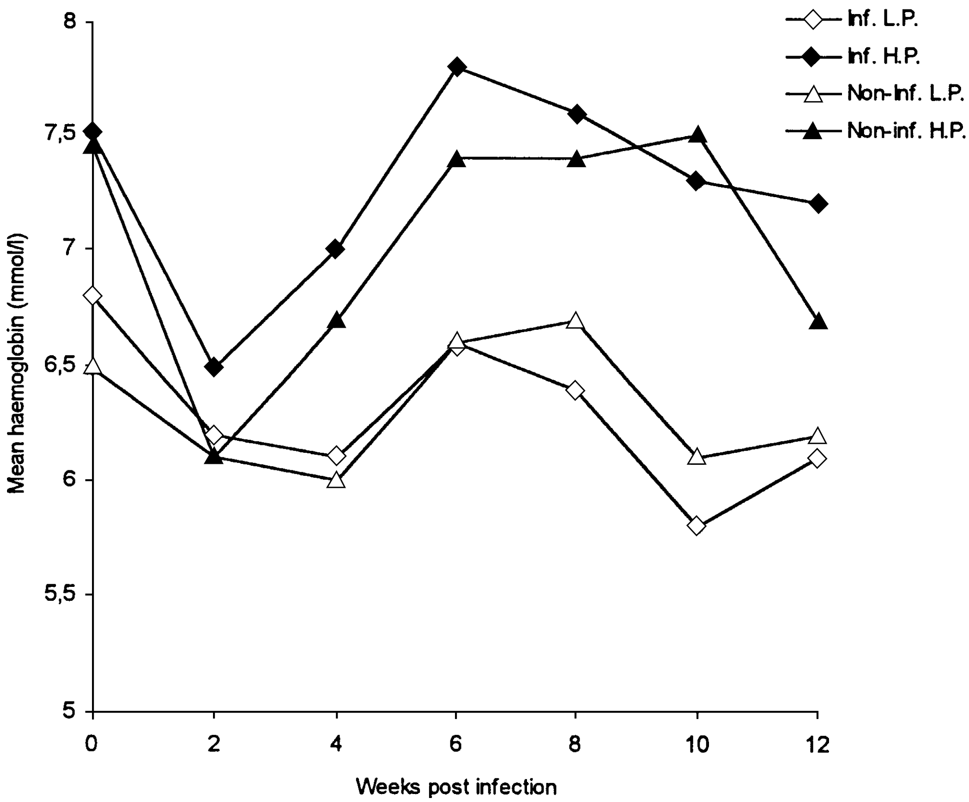

Fig. 3. Mean haemoglobin concentration of blood from *Schistosoma japonicum* and *Trichuris suis*-infected pigs. Inf. L.P.: infected low-protein group; Inf. H.P.: infected high-protein group; Non-inf. L.P.: non-infected low-protein group; Non-inf. H.P.: non-infected high-protein group. Significant group differences were seen week 0 ($P < 0{\cdot}001$) and 4–12 weeks post-infection: Inf. L.P. and Non-inf. L.P. < Inf. H.P. and Non-inf. H.P. ($P < 0{\cdot}05$).

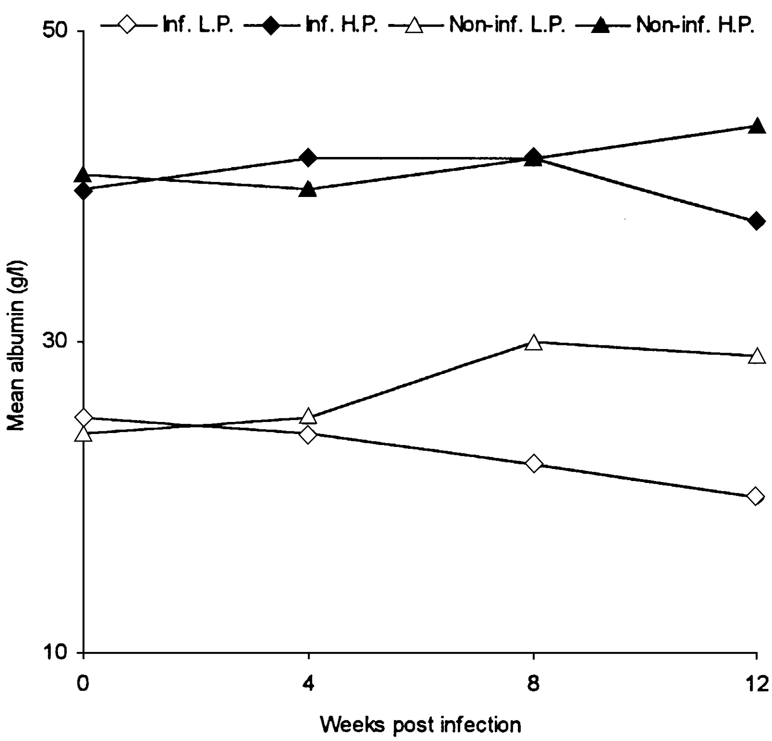

Fig. 4. Mean albumin concentration of serum from *Schistosoma japonicum* and *Trichuris suis*-infected pigs. Inf. L.P.: infected low-protein group; Inf. H.P.: infected high-protein group; Non-inf. L.P.: non-infected low-protein group; Non-inf. H.P.: non-infected high-protein group. Significant group differences were seen weeks 0–12 (Inf. H.P. and Non-inf. H.P. > Inf. L.P. and Non-inf. L.P.; $P < 0{\cdot}01$) and 8 and 12 weeks post-infection: (Non-inf. L.P. > Inf. L.P. ($P < 0{\cdot}01$).

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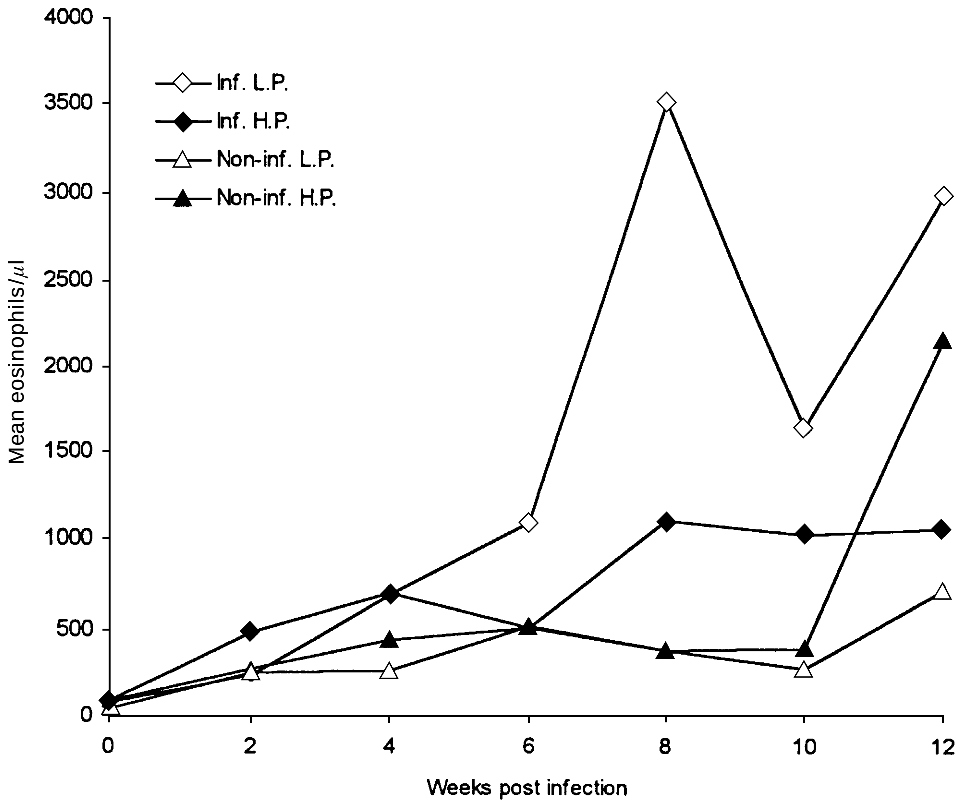

Fig. 5. Mean eosinophil counts of blood from *Schistosoma japonicum* and *Trichuris suis*-infected pigs. Inf. L.P.: infected low-protein group; Inf. H.P.: infected high-protein group; Non-inf. L.P.: non-infected low-protein group; Non-inf. H.P.: non-infected high-protein group. Significant group differences were seen week 4 (Inf. L.P. and Inf. H.P. > Non-inf. L.P. and Non-inf. H.P.; $P < 0{\cdot}05$) and 8 and 10 weeks post-infection: (Inf. L.P. > Non-inf. L.P. and Non-inf. H.P. ($P < 0{\cdot}05$).

anaemia in the low-protein group (ANOVA on repeated measures, $P < 0{\cdot}001$).

A significant hypoalbuminaemia was observed in the 2 low-protein groups from the time of infection ($P < 0{\cdot}001$) onwards as compared to the high protein-groups (Fig. 4). However, in the infected low-protein group the hypoalbuminaemia became more severe from 4 weeks post-infection onwards, and from week 8 the mean albumin values were significantly lower than those of the non-infected low-protein group ($P < 0{\cdot}01$). In the infected high-protein group the albumin level showed a similar decrease towards the end of the study, but statistically no difference was seen at the end of the study between the 2 high-protein groups. Both the low-protein diet and the parasite infections were found to be causing factors for hypoalbuminaemia, though the most significant factor was the low-protein diet (ANOVA on repeated measures, $P < 0{\cdot}001$).

The eosinophil counts were very similar in all 4 groups at the time of infection and 2 weeks after the infections (Fig. 5). At week 4 post-infection, the eosinophil counts in the 2 infected groups were significantly higher than the non-infected groups ($P < 0{\cdot}05$), and that pattern continued in the infected low-protein group throughout the study (significantly higher at weeks 8 and 10 post-infection, $P < 0{\cdot}05$). The ANOVA on repeated measurements revealed that only the parasite infections and not diet treatment were significantly associated with high eosinophil counts ($P < 0{\cdot}01$).

DISCUSSION

The results from the present study demonstrated that a low-protein diet given to young growing pigs for 11 weeks before infection aggravated a *S. japonicum* infection by giving rise to higher worm establishment and higher faecal and liver egg counts as compared with pigs kept on a high-protein diet. The establishment rate and fecundity of *T. suis* appeared to be only marginally affected by the low protein-diet. However, definitive conclusion for this parasite can not be drawn since worm expulsion from the intestine had started before the study ended and a possible effect of the diet on the expulsion can not be excluded. Clarke (1968) showed that a low-protein diet given to *Nippstrongylus brasiliensis*-infected rats resulted in expulsion of the worms at an earlier date than normal and van Houtert *et al.* (1995) found that a protein-supplemented diet given to sheep infected with *Trichostrongylus colubriformis* increased the expulsion rate of the worms.

In the present study changes in eosinophil counts were directly related to the parasite infections and were significantly increased in infected pigs, whereas

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the haemoglobin levels and the body weights were significantly related to diet treatment and not to the parasite infections. Hypoalbuminaemia was strongly associated with the low-protein diet but was additionally associated with the parasite infections, reflected in significantly aggravated hypoalbuminaemia in the infected low-protein pigs compared to the other groups. This is in line with a recent study by Willingham *et al*. (1997) which showed that the serum albumin concentration in well-nourished pigs was not significantly affected by a *S. japonicum* infection (150 cercariae/kg body weight). The fact that body weight and haemoglobin levels were not found to be significantly associated with parasitism in the present study is also in accordance with the study by Willingham *et al*. (1997*b*) and may be a consequence of the relatively light infections of the pigs. In contrast to our findings, Yason & Novilla (1984) found severe clinical signs, coincident with egg production, in young pigs infected with a single high (5000–6000) cercarial dose. Diet was not mentioned in their study but pigs received 400 cercariae/kg body weight while in our study the pigs were given approximately 50 cercariae/kg body weight (low-protein pigs) and 25 cercariae/kg body weight (high-protein pigs) or only 1/8–1/16 the dose/unit body weight. Robinson & Lewert (1980) showed in an experimental study with *S. japonicum*-infected rabbits, that rabbits developed anaemia during early patency. Coutinho *et al*. (1992) concluded that heavy infections of *S. mansoni* worsened the nutritional status of the undernourished host in an experimental study of mice fed a low-protein- and energy-diet.

One of the limitations in the present study was the lack of control pigs harbouring single infections with either *S. japonicum* or *T. suis* which makes it difficult to draw more specific conclusions. Why the establishment rate of *S. japonicum* was so low in the present study remains unclear but one could speculate that the *T. suis* infection had had a negative influence on the establishment of *S. japonicum*. With respect to the clinical parameters of the pigs probably both parasite species have contributed to the picture seen.

Reviews of human studies have shown that schistosomiasis and soil-transmitted helminths often are associated with malnutrition (Stephenson, 1993). Studies in endemic communities have shown that weight gain and normalization of haemoglobin levels may result from treatment of undernourished children infected with *S. japonicum*, *T. trichiura* and other nematodes (Stephenson *et al*. 1989; Hadju *et al*. 1996; McGarvey *et al*. 1996).

These data suggest that the success of establishment of *S. japonicum* is associated with the nutritional status of the definitive host but, as indicated from human studies, schistosomiasis may exacerbate malnutrition in the host. Hence, it appears that a vicious circle exists, whereby host malnutrition *per se* increases the chances for the parasite to establish in larger numbers and consequently heavier parasite infections may further deplete the host's nutritional reserves. Future studies should consider different types of malnutrition in relation to *S. japonicum* alone and in combination with other parasites found in endemic areas with emphasis on the mechanisms by which parasites and host malnutrition affect each other over time.

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