

## SCHISTOSOMA JAPONICUM IN THE PIG: THE HOST-PARASITE RELATIONSHIP AS INFLUENCED BY THE INTENSITY AND DURATION OF EXPERIMENTAL INFECTION

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**Abstract.** Parasitologic, clinicopathologic, and pathologic aspects of *Schistosoma japonicum* infections of varying durations and intensities were studied in growing pigs injected intramuscularly with a dose of either 0, 100, 500, or 2,000 cercariae and killed at 4, 11, 17, or 24 weeks postinfection (PI). The number of viable worm pairs decreased significantly in the high dose group after 11 weeks PI but not in the lower dose groups; however, a stable population of immature worms persisted throughout the study in all dose groups. Liver egg counts also tended to decrease in the high dose group after 11 weeks but not in the other groups. Fecal egg excretion began at six weeks PI, was highest at eight weeks PI with a pronounced peak occurring only in the high dose group, and then decreased to low levels by 14 weeks PI in all groups. Egg counts from the feces as well as the liver correlated strongly with worm pair numbers during the acute phase of infection. The only clinicopathologic abnormality observed was an increase in circulating eosinophils corresponding to cercarial dose in all infected pigs by week six with peak counts occurring between six and eight weeks PI. The pigs exhibited no clinical signs of disease aside from diarrhea at the onset of patency. However, lesions were present throughout the large intestine of all infected pigs from 11 weeks PI, gradually decreasing with time. Severe liver fibrosis occurred in the 500 and 2,000 dose groups mainly at 11 weeks PI and then decreased in severity. In the liver, but not in the intestine, the severity of lesions at all time points was proportional to the cercarial dose given. The results indicate that after several weeks of patency, pigs with high intensities of *S. japonicum* infection are able to effectively eliminate the majority of adult worms while maintaining a stable population of immature schistosomes.

Important studies on the biology of schistosomes that infect humans have been conducted using laboratory rodents and primates as experimental hosts. However, the validity and relevance of the results have been questioned, even by the responsible researchers themselves, since in most instances these animals are not natural hosts of the parasites.<sup>1,2</sup> With regard to *Schistosoma japonicum*, only a few experimental investigations into natural definitive host-parasite relationships have been published despite the fact that many mammalian species, including domestic animals, serve as reservoir hosts of this zoonotic trematode.<sup>3,4</sup> Pigs are a significant reservoir host of *S. japonicum* that can transmit the disease to humans and other animals,<sup>5–11</sup> and their own growth and reproductive capacity is lowered due to disease-related morbidity and mortality.<sup>5,6</sup> Moreover, pigs are economically important livestock in *S. japonicum*-endemic countries of east Asia and therefore a parasitic disease affecting swine production has great agricultural relevance. Recognition of the many biological similarities between pigs and humans has recently led to investigation of their use as a host model for *S. japonicum* infection.<sup>12–15</sup> Experimental studies on porcine schistosomiasis japonica may thus provide important information about the disease in both pigs and humans.

The objective of the present study was to investigate the host-parasite relationship of *S. japonicum* infection in pigs by studying experimental infections of differing intensities at various intervals following infection. We accomplished this by sequentially slaughtering subgroups of pigs infected with three different cercarial doses and assessing worm burdens, fecal egg excretion, and tissue egg accumulation. In addition, clinicopathologic and pathologic parameters of these three groups and a group of uninfected pigs were examined. Only the gross pathologic changes of the main tar-

get organs, the large intestine and the liver, are reported here. Other pathologic and histopathologic observations will be presented in a separate paper.

### MATERIALS AND METHODS

**Experimental animals and design.** Ninety-six helminth-naive, specific pathogen-free Danish Landrace/Yorkshire/Duroc cross-bred pigs, initially 6–10-weeks old, with an average weight of 15.7 kg, were used for the experiment. They were housed together under helminth-free conditions and fed a standard ration of ground barley with water provided ad libitum throughout the study. The pigs, 46 females and 50 castrated males, were allocated according to sex, weight, and litter origin into four groups of 24 pigs. They were infected by intramuscular injection with Iscoves medium-suspended cercariae of an *S. japonicum* isolate originating from Anhui Province, People's Republic of China, which is maintained at the Danish Bilharzias Laboratory in *Oncomelania hupensis hupensis*.<sup>15</sup> One group of pigs served as uninfected controls and received an injection of plain suspension medium while the other three groups were infected with either 100, 500, or 2,000 cercariae per pig. These four groups were further divided into four subgroups of six pigs each, also based on sex, weight, and litter origin. One subgroup from each group was killed and perfused at 4, 11, 17 and 24 weeks postinfection (PI), respectively. Fecal samples were collected for parasite egg counts every two weeks from the rectum of all infected pigs. In addition, pigs in the four subgroups that were killed at 24 weeks PI were weighed and had their blood sampled for clinicopathologic examination every two weeks during the investigation. The pigs used in this experiment were treated in accordance with animal ethics laws of Denmark.

**Parasitology.** Schistosomes were recovered from the liver and intestinal mesenteries using the perfusion technique of Johansen and others<sup>16</sup> modified to selectively perfuse the specified organs.<sup>17</sup> Pigs were given praziquantel (50 mg/kg) orally 1 hr prior to perfusion to ensure hepatic shift of perfusable schistosomes, and then sedated 30 min later with an intramuscular injection of azaperonum (4 mg/kg). Animals were killed by intravenous administration of pentobarbital (30 mg/kg) following intravenous injection of heparin (500 IU/kg) via an indwelling catheter placed in an auricular vein.

The perfusion fluid used was a sodium citrate buffer (15 g of sodium citrate plus 8.5 g of sodium chloride/L of water) to which was added the vasodilator sodium nitroprusside (10 ml/L of a solution of 450 mg sodium nitroprusside/L of isotonic glucose). Collection of perfusion fluid was accomplished by inserting into the portal vein a plastic tube to which a 1-m rubber hose was attached. The other end of the tube was directed into a 45- $\mu$ m sieve to collect worms from the fluid. The worms were counted according to sex and maturity. Pigs were perfused in random order at each of the times of killing. Subsequent to perfusion, the entire intestinal tract of each pig was examined for residual adult schistosomes, the number, sex, viability, and location of which were recorded. The percentage worm establishment was calculated by dividing the number of total worms recovered from each pig by the number of cercariae with which they were injected.

The number of schistosome eggs in the liver was determined by digesting a 5-g sample of the left lateral hepatic lobe in 3% KOH at 37°C for 18 hr.<sup>18</sup> Egg counts of three 1-ml aliquots of the digestion fluid were determined using Sedgwick-Rafter Cell S50 counting chambers (Graticules Limited, Tonbridge, United Kingdom). The mean of these three counts was then used to calculate the number of eggs per gram of liver (EPG liver).

Fecal egg counts were determined for all infected pigs in each group still alive at each sampling time point. Schistosome eggs in the feces were quantified using a method combining filtration and sedimentation/centrifugation techniques.<sup>19,20</sup> Briefly, a 5-g sample of feces was washed with 1.2% saline through a series of three sieves with mesh sizes of 400  $\mu$ m, 100  $\mu$ m, and 50  $\mu$ m, respectively. The material collected in the 50- $\mu$ m sieve was washed with 1.2% saline, sedimented twice, and the resulting sediment was centrifuged. One-fifth of the sediment was removed with a pipette and examined microscopically for schistosome eggs with the resulting egg count equivalent to eggs per gram of feces (EPG feces). In addition, feces from all pigs were examined for other gastrointestinal helminths by the McMaster technique<sup>21</sup> at both the beginning and end of the experiment. The fecundity of worms was estimated by relating the EPG liver and EPG feces at certain time points to the number of worm pairs recovered.

**Clinical pathology.** Clinicopathologic parameters were assessed using standard procedures. Packed cell volume (PCV) was determined using a microhematocrit centrifuge and hemoglobin concentration was determined by automated spectrophotometric methods. Erythrocyte and total leukocyte counts were determined using a Coulter counter. The number of circulating eosinophils was enumerated following staining with eosin using Burker-Turk (Scherf Prazision, Ostheim,

Urspringen, Germany) counting chambers. Albumin concentration in the serum was assessed by a bromocresol green method using a spectrophotometer (Cobas Fara; Roche, Basel, Switzerland).

**Gross pathology.** Following perfusion, the organs were removed for pathologic examination. The entire length of the large intestine was opened for inspection and palpation of lesions and the overall degree of pathologic change was scored as none = 0; mild (up to 15 focal lesions in the entire large intestine) = 1; moderate (16–30 focal lesions) = 2; or severe (more the 30 focal lesions) = 3. The liver was inspected and palpated and lesions were graded as mild (a few nodules and/a slight increase in interlobular connective tissue) = 1; moderate (moderate numbers of nodules and/or a moderate increase in interlobular connective tissue) = 2; or severe (large numbers of nodules and/or a marked increase in interlobular connective tissue) = 3. Mesenteric and portal lymph nodes were examined. Group means of scores were calculated for the large intestine and liver.

**Statistical analyses.** One-way analysis of variance was used to test for differences in group means for hemoglobin levels, PCV, erythrocyte and leukocyte counts, serum albumin levels, weights, worm burdens, percent worm recovery, and fecal and liver egg counts. Eosinophil counts were  $\log_{10}(x)$  transformed while worm burdens, percent worm recovery, and fecal and liver egg counts were transformed to  $\log_{10}(x + 1)$  before being tested for differences between groups and timepoints. A posteriori pairwise comparisons of means were done using Scheffe's range test. The relationships between numbers of worm pairs, fecal egg counts at the time of maximum egg excretion, and liver egg counts were investigated with Pearson's correlation test. For all tests,  $P$  values < 0.05 were considered significant.

## RESULTS

Infections were obtained in all pigs given cercariae. Approximately 15–20% of the pigs in each infected group developed diarrhea at 6–8 weeks PI, but no other clinical signs suggestive of schistosomiasis were observed. No significant differences in weight gain were observed between infected and noninfected control pigs during the study. There were considerable variations between animals within groups with respect to fecal egg excretion, worm burden, and liver egg counts. All pigs, including uninfected controls, harbored infections with the ciliate protozoan *Balantidium coli* throughout the study, which was detected during coprologic examination for schistosome eggs. However, none of the pigs excreted ova of other helminths during the study nor were any found to harbor helminths other than schistosomes at the time of necropsy.

**Worm recovery.** The percentage worm establishment tended to be lower throughout the study for the 500 dose group than for the other two dose groups but no significant differences were found between the three groups at any time point (Figure 1). Total worm burdens for the subgroups are presented in Table 1. Although worm burdens in each dose group tended to decrease with time, no significant statistical differences were found within groups between the different time points. Male-to-female worm ratios ranged from 0.7 to

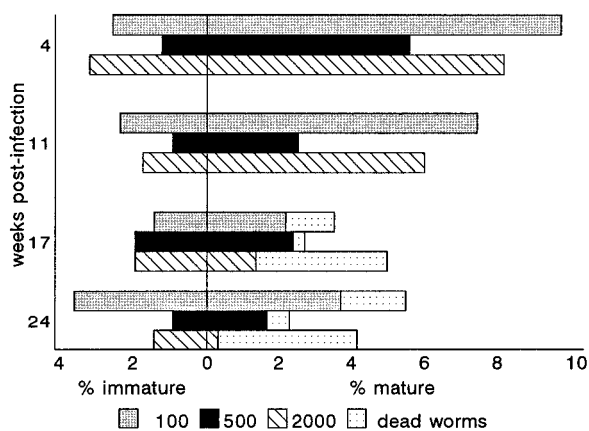


FIGURE 1. Worm recovery (geometric means), i.e., percentage of the infection dose recovered as mature (right of zero) or immature worms (left of zero) presented as a function of time for the three groups of pigs infected with different doses of *Schistosoma japonicum*. The percentage of adult worms considered dead at the time of perfusion is also indicated.

1.2 in the different subgroups with no significant differences between or within groups at the different time points.

Immature worms were recovered from pigs in all three groups throughout the study (Figure 1). The number of immature worms as a proportion of the total worm burdens remained fairly stable for each group throughout the study with the mean for the 12 subgroups being 28% (range = 18–43%). No significant statistical differences in the percentage establishment of immature worms between or within groups at any time point were found. These long, slender immature worms could not be sexed because neither gonads nor any other internal organs were identifiable. Most of the pigs harboring these immature worms had slightly more adult female than male worms.

The vast majority of worms still present in the vasculature after perfusion were found pinned in the mesenteric veins throughout the large intestine. The exact location of these worms in the vessels of the large intestine appeared to be independent of duration and intensity of infection and varied greatly between individual pigs. Less than 4% of the residual worms were located in the small intestinal veins. At 4 and 11 weeks PI, the number of residual worms was low compared with the number of worms recovered by perfusion and were viable. However, very few adult worms were recovered by perfusion from the 2,000 dose group pigs at 17 weeks PI and from any of the pigs in the three groups at 24 weeks PI (Table 1). Most residual worm pairs at these time points, however, were dead and decaying (Figure 1) and embedded in firm nodules, preventing their removal in toto.

These dead worms are included in figures for total worm burdens and percentage worm establishment. We also determined the number of viable adult worm pairs (excluding dead and immature worms) at the different timepoints (Table 1). The number of viable adult worm pairs at four weeks PI differed significantly among the three infected groups, with the 2,000 dose group having a significantly higher number than the other two groups and the 500 dose group having a significantly higher number than the low dose group. At 11 weeks PI, the number of viable worm pairs was significantly

TABLE 1

Number of *Schistosoma japonicum* worms and viable adult worm pairs recovered from pigs in the three dose groups at different timepoints\*

Perfusion time	Cercarial dose/pig		
	100	500	2,000
<b>Total worm burdens</b>			
4 weeks PI	11.9 (7.7–18.0)	33.0 (21.5–50.5)	219.9 (124.3–388.6)
11 weeks PI	9.5 (3.2–25.5)	16.5 (5.7–44.4)	150.9 (90.9–250.0)
17 weeks PI	4.8 (0.7–18.8)	22.5 (10.3–47.9)	133.4 (72.7–244.0)
24 weeks PI	8.8 (5.6–13.4)	15.7 (9.2–26.3)	107.8 (67.4–172.1)
<b>Viable adult worm pairs</b>			
4 weeks PI	4.4 (2.9–6.6)	12.9 (8.6–19.1)	75.5 (43.4–130.9)
11 weeks PI	3.2 (1.0–7.9)	4.8 (2.0–10.1)	56.7 (35.6–90.0)
17 weeks PI	1.3 (0.6–4.0)	4.6 (1.7–10.8)	6.0 (3.5–9.7)
24 weeks PI	1.4 (0.3–3.2)	2.7 (1.2–5.3)	2.1 (0.7–4.8)

\* Values are geometric means (95% confidence intervals). PI = postinfection.

higher in the 2,000 dose group than in both other groups, but at 17 weeks PI it was only significantly higher than in the 100 dose group. At 24 weeks PI, very few viable worms were recovered from the high dose pigs and there was no significant difference in the number of viable worm pairs recovered from the three groups. The number of viable worm pairs recovered from the subgroups dosed with 2,000 cercariae were significantly lower at 17 and 24 weeks PI than at 4 and 11 weeks PI. No significant differences in numbers of viable worm pairs were found between the different time points in the two lower dosed groups although dead worms were a component of their worm populations at 17 and 24 weeks PI.

**Tissue egg counts.** The tissue egg counts of the liver in the various groups of infected pigs at the different time

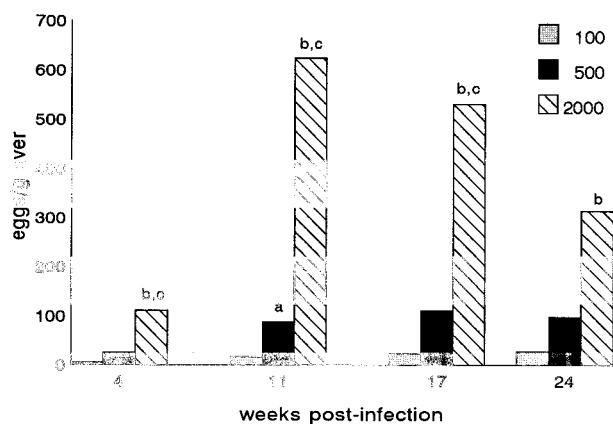


FIGURE 2. Number of *Schistosoma japonicum* eggs/g recovered from pig liver samples (geometric means) as a function of time for the three dose groups (a = 500 versus 2,000 dose group at 11 weeks postinfection [PI];  $P < 0.05$ ; b = 2,000 versus 100 dose group at all time points;  $P < 0.05$ ; and c = 2,000 dose group at four weeks PI versus 11 and 17 weeks PI;  $P < 0.05$ ).

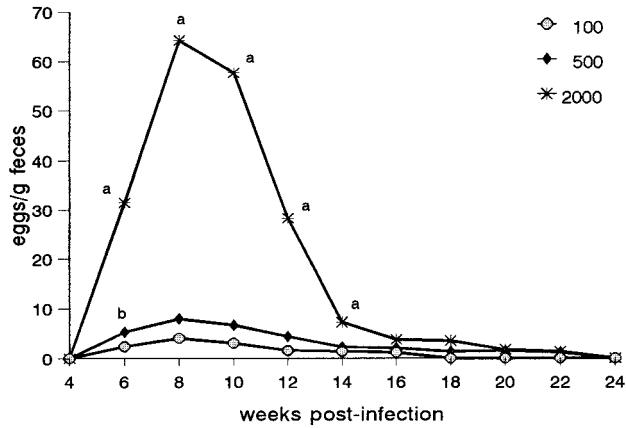


FIGURE 3. Fecal egg excretion expressed in eggs/g of feces (geometric means) for the three groups of pigs infected with different doses of *Schistosoma japonicum* (a = 2,000 versus 100 and 500 dose groups at 6–14 weeks postinfection [PI];  $P < 0.05$ ; b = 500 versus 100 dose group at 6 weeks [PI];  $P < 0.05$ ).

points of the study are shown in Figure 2. Tissue egg counts for the three groups differed significantly from each other at 11 weeks PI. In addition, tissue egg counts of the 2,000 dose group were significantly higher than those of the 100 dose group at four, 17, and 24 weeks PI, and the mean tissue egg count of the 2,000 dose group was significantly lower at four weeks PI than at 11 and 17 weeks PI.

**Fecal egg excretion.** The dynamics of fecal egg excretion in the three infected groups of pigs are shown in Figure 3. All pigs in the 2,000 dose group and most pigs of the other infected groups excreted *S. japonicum* eggs at week six PI. In six pigs (four in the 100 dose group and two in the 500 dose group), eggs were not detected until eight weeks PI. The increase and decrease in egg excretion was also much more rapid and pronounced in the highest dosed group. Egg output of the three groups differed significantly from each other at six weeks PI. Thereafter, fecal egg excretion of the 2,000 dose pigs was significantly higher than that of the two lower dosed groups until week 16 PI and afterwards, when very few eggs were excreted by any of the pigs. However, differences in fecal egg excretion in the 100 and 500 dose groups were not significant at any time point after six weeks PI.

**Worm fecundity.** The number of EPG liver per worm pair at four and 11 weeks PI was used as one measure to compare worm fecundity of the three dose groups (Table 2). No statistically significant difference was observed between the mean EPG liver per worm pair of the three groups at these time points, although the mean EPG liver per worm pair at 11 weeks PI was approximately 2–3 times higher for the 500 cercariae dose group than for the other two groups.

The number of EPG feces per worm pair was used to assess fecundity for those pigs that were perfused at 11 weeks PI using their fecal egg counts at week 10. The fecal egg counts at this particular time were chosen since they could be related to worms recovered very soon thereafter and because very few or no eggs were detected in the feces of pigs at later time points. Comparison of the EPG feces at 10 weeks PI per worm pair indicated no significant differences between the three groups.

**Correlations.** The numbers of eggs excreted in the feces

TABLE 2

Fecundity estimates at four and 11 weeks postinfection (PI) for the three groups of pigs infected with different doses of *Schistosoma japonicum*\*

Cercarial dose	Worm pairs	Liver EPG/worm pair	Fecal EPG/worm pair†
4 weeks PI			
100	4.4 (2.9–6.6)	1.5 (0.1–4.5)	NA
500	12.9 (8.6–19.1)	2.2 (0.5–5.8)	NA
2,000	75.5 (43.4–130.9)	1.6 (0.5–3.6)	NA
11 weeks PI			
100	3.2 (1.0–7.9)	5.7 (1.6–16.2)	1.0 (0.1–2.7)
500	4.8 (2.0–10.1)	19.8 (8.6–44.1)	1.4 (0.4–3.0)
2,000	56.7 (35.6–90.0)	11.1 (8.1–15.0)	0.7 (0.3–1.4)

\* Values are geometric means (95% confidence intervals). EPG = eggs per gram.

† Based on week 10 fecal egg counts of those pigs perfused at week 11. NA = not available.

and accumulated in the liver were compared with the number of worm pairs recovered at four and 11 weeks PI (Table 3). A strong correlation was found between the number of worm pairs recovered from groups killed at four and 11 weeks PI and the liver egg counts of these pigs ( $P < 0.001$ ) and also between the numbers of worm pairs recovered from pigs killed at 11 weeks and the fecal egg counts at eight and 10 weeks PI ( $P < 0.001$ ). In addition, these fecal egg counts also correlated strongly with liver egg counts of the group of pigs killed 11 weeks PI ( $P < 0.001$ ).

**Clinical pathology.** Blood eosinophilia was observed in the 2,000 dose group after four weeks PI (Figure 4), peaking between six and eight weeks PI when their numbers of eosinophils differed significantly from those of the uninfected control group. Eosinophil counts for the 500 and 100 dose groups at these time points tended to be higher (but not significantly) than those of the control group. An elevation of eosinophil counts was observed in all groups, including the uninfected controls, between 18 and 22 weeks PI.

No significant differences between infected and control pigs were observed at any time during the study with regard to hemoglobin and albumin levels, PCV, and erythrocyte and total leukocyte counts.

TABLE 3

Coefficients of the linear correlations between recovered worm pairs, fecal egg excretion, and liver tissue egg counts\*

Variables†	Correlation coefficient
4 weeks PI	
WP/LTEC	0.81
11 weeks PI	
WP/LTEC	0.95
WP/FEC8	0.80
WP/FEC10	0.85
FEC8/LTEC	0.77
FEC10/LTEC	0.84

\*  $P$  values for all correlations were  $<0.0001$ . PI = postinfection.

† WP = worm pairs; LTEC = liver tissue egg counts; FEC8 = fecal egg count at week 8 PI; FEC10 = fecal egg count at week 10 PI.

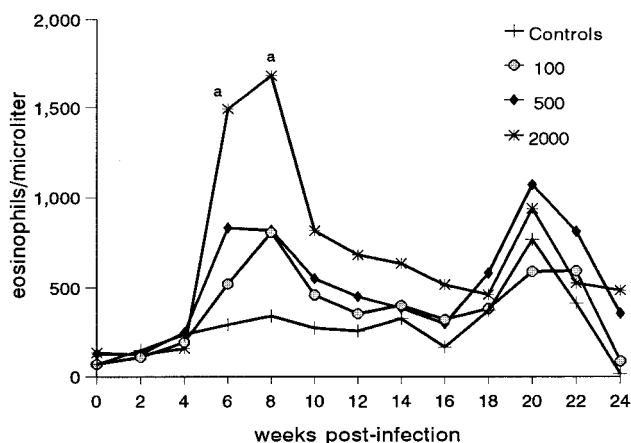


FIGURE 4. Eosinophil counts (geometric means) for pigs infected with the three different doses of *Schistosoma japonicum* and uninfected control pigs (a = 2,000 dose group versus control group at 6–8 weeks postinfection [PI];  $P < 0.05$ ).

**Gross pathology.** At four weeks PI, no gross pathologic changes were found in the large intestine. At 11, 17 and 24 weeks PI, the predominant lesions in all dose groups were focally distributed petechial hemorrhages and small hyperemic foci (< 3 mm in diameter) in the mucosa in any part of the large intestine. The lesions were sometimes distinctly localized along the course of blood vessels. Two pigs at both 11 and 17 weeks PI, respectively, showed ecchymotic hemorrhages (Figure 5) and even more extensive hemorrhages, and at 17 weeks PI some of the hemorrhagic areas were ulcerated.

Intestinal lesions occurred in all 18 infected pigs at 11 weeks PI, in 16 of 18 pigs at 17 weeks PI, and in 11 of 18 pigs at 24 weeks PI, with only slight differences in frequency between the dose groups. Lesions were most severe in the 2,000 dose group, while the 100 and 500 dose groups had mild or moderate lesions. A reduction in severity was found at 17 weeks PI in the 100 and 2,000 dose groups, but in the 500 dose group lesions were more pronounced at this stage. At 24 weeks PI, the lesions were generally mild and in the

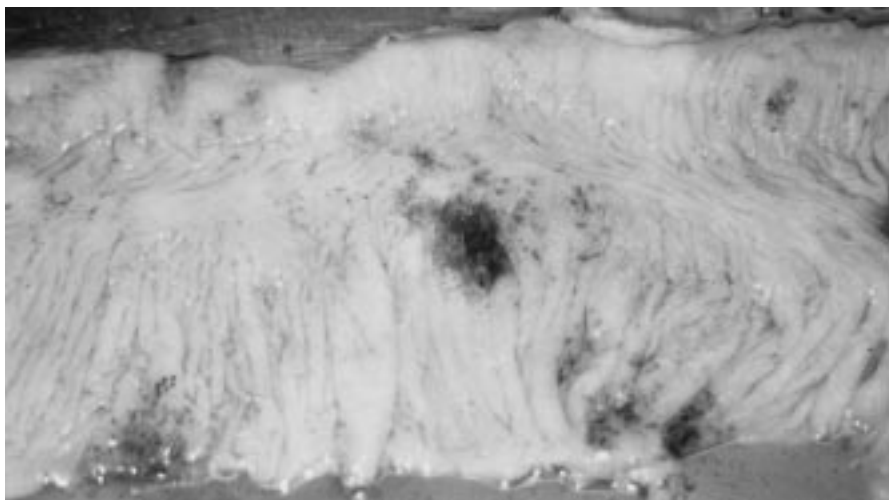


FIGURE 5. Proximal colon of a pig in the 2,000 dose group at 17 weeks postinfection showing focal petechial and ecchymotic hemorrhages.

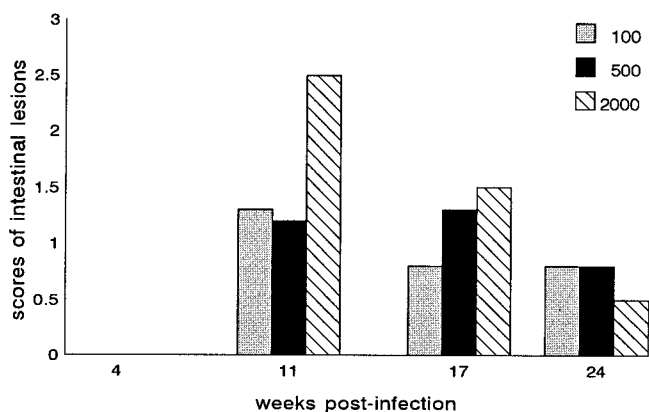


FIGURE 6. Mean scores of intestinal lesions in the different groups of pigs at the different timepoints. A score of 0 = none; 1 = mild; 2 = moderate; and 3 = severe lesions.

pigs in the 2,000 dose group the intestine was less affected than in those of the two lower dose groups (Figure 6).

In the 2,000 dose group at 17 weeks PI and in all groups at 24 weeks PI, mesenteric veins throughout the large intestine contained numerous rice grain-sized, gray-white nodules with dead worms or worm remnants. The latter were identifiable by the presence of black schistosomal pigment, i.e. a pigment derived from the vitelline glands and intestine of female worms.<sup>22</sup> In the submucosa, similar nodules, sometimes with overlying mucosal ulceration, were found in the cecum and colon of a few pigs at 11, 17, and 24 weeks PI. Hyperplasia of the gut associated lymphoid tissue of the large intestine was a prominent feature in one 500 dose group at 11 weeks PI, in one 2,000 dose group pig at 17 weeks PI, and in three 2,000 dose group pigs at 24 weeks PI, while enlargement of mesenteric lymph nodes was marked only in two pigs (one each in the 500 and 2,000 dose groups) at 24 weeks PI.

Gross pathologic lesions of the liver were found in all dose groups at all time points (Table 4). They consisted of disseminated, small, firm, gray-white nodules on the liver surface and within the parenchyma and a multifocal or gen-

TABLE 4

Frequency and type of liver lesions in the three dose groups at different timepoints

Cercarial dose*	No. of pigs with lesions	Lesions		
		Disseminated nodules	Multifocal fibrosis	Generalized fibrosis
4 weeks PI				
100	1/6	1	1	0
500	2/6	1	1	1
2,000	3/6	0	1	2
Total	6/18	2	3	3
11 weeks PI				
100	6/6	2	6	0
500	6/6	2	0	6
2,000	6/6	2	0	6
Total	18/18	6	6	12
17 weeks PI				
100	5/6	3	1	4
500	6/6	5	1	5
2,000	6/6	3	1	5
Total	17/18	11	3	14
24 weeks PI				
100	4/6	1	1	3
500	6/6	2	3	3
2,000	6/6	1	2	4
Total	16/18	4	6	10

\* PI = postinfection.

eralized increase in interlobular connective tissue (Figure 7). Typical pipestem fibrosis was not observed. Severe liver fibrosis was seen only in the 500 and 2,000 dose groups and occurred with the highest frequency at 11 weeks PI, after which there was a gradual reduction in severity at 17 and 24 weeks PI. Severe fibrosis was often accompanied by enlargement of portal lymph nodes and occasionally by ascites. The 100 dose group generally had mild lesions, although two pigs had moderate fibrosis at 17 weeks PI, after which the severity of lesions in this group also decreased. The degree of grossly visible hepatic fibrosis increased with the number of cercariae given and also tended to correlate with the number of eggs in the liver tissue in each dose group (compare Figures 2 and 8). However, the liver lesions in the 500 dose group were more severe than was expected from the relatively low tissue egg counts in this group.

#### DISCUSSION

The data presented show that Danish Landrace/Yorkshire/Duroc cross-bred pigs are able to mount an effective regulatory response to high intensities of infection with *S. japonicum* resulting in the elimination of adult worms. After several weeks of patent infection the number of eggs in the feces decreased dramatically in the high dose group and more gradually in the lower dose groups, resulting in no eggs being detected in the feces of any pigs at the end of the study. In addition, recovery of viable adult worms decreased greatly in the 2,000 dose group after 11 weeks PI so that by the end of the study there were no significant differences in worm burdens of the three groups. Liver egg counts tended to decrease after 11 weeks of infection in the



FIGURE 7. Liver of a pig in the 500 dose group at 11 weeks postinfection showing disseminated white nodules and severe generalized fibrosis.

2,000 dose group while remaining stable in the 500 and 100 dose groups. Also, related pathologic lesions in the three groups decreased or remained unchanged in severity during the study.

Albino rats, rhesus monkeys, and water buffaloes are also known to regulate experimental *S. japonicum* infection following different time periods. Rats have been found to eliminate schistosomes by five weeks PI with the schistosomes never leaving the liver and never reaching maturity,<sup>3,23</sup> whereas rhesus monkeys develop patent infections with egg excretion and worm burdens decreasing gradually to low levels over several months.<sup>24,25</sup> Water buffaloes, an important natural host of *S. japonicum*, also develop patent infections approximately six weeks PI (infection dose of 3,000 cercariae) and excrete large numbers of eggs initially, with the number gradually decreasing so that few eggs are detected after 16–20 weeks PI and none by 27 weeks PI, when adult worms are no longer recovered.<sup>3,4</sup> Thus, it appears that the pig undergoes recovery from single high-dose infections with *S. japonicum* in a manner similar to the water buffalo.

Despite the elimination of adult worms in the high dose group, a stable population of immature worms remained throughout the 24-week study period in all three dose groups. A similar finding occurred in one of our previous

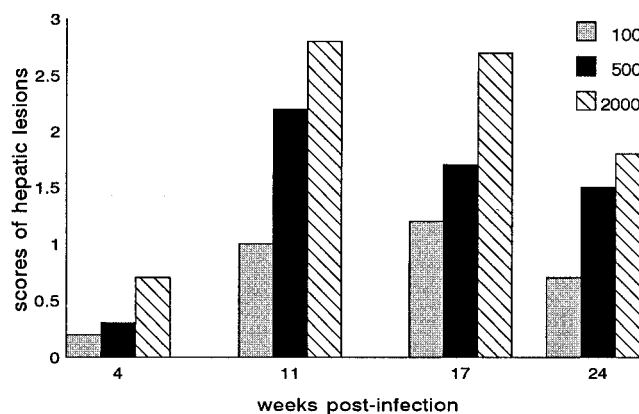


FIGURE 8. Mean scores of hepatic lesions in the different groups of pigs at the different timepoints. A score of 0 = none; 1 = mild; 2 = moderate; and 3 = severe lesions.

experiments in which pigs were infected with various doses by a percutaneous leg immersion technique and perfused 17 weeks later (Willingham AL, unpublished data), and has also been observed in experimental canine and murine studies.<sup>26,27</sup> As in the canine experiments,<sup>26</sup> we were not able to sexually differentiate these worms because it was impossible to discern any of their internal organs. One possible explanation for the presence of these worms is that they were females left unpaired that remained immature due to a shortage of male worms since most of the pigs with immature worms had adult male-to-female ratios less than or equal to one. However, in one murine study, juvenile worms were recovered from mice that harbored excess adult males.<sup>27</sup> Another explanation could be that they were worms of both sexes that remained undeveloped due to suppressive effects of either early establishing schistosomes or of the host reaction to establishment of adult schistosomes and/or oviposition.

The observed numbers of schistosomes in this study was lower than expected based on our previous investigations using the intramuscular injection method of infection.<sup>15</sup> However, the results are comparable with those of other experimental studies using People's Republic of China isolates in pigs,<sup>3,12,28</sup> all of which recorded less than 15% recovery from the infection dose. It has been shown that higher establishment occurs when mice are infected with either the Japanese or Philippine strains of *S. japonicum* as opposed to isolates from the People's Republic of China.<sup>29</sup> Recovery of 19–30% was obtained in an experimental study in pigs using a Philippine strain.<sup>30</sup>

Egg numbers in the feces and livers varied tremendously among pigs in all groups. There was no significant difference in the number of fecal eggs per worm pair among the three groups at 11 weeks PI, indicating no crowding effect with the worm burdens established. The decrease in the number of eggs excreted in the pigs' feces starting approximately 4–6 weeks after patency was possibly due initially to reduced worm fecundity but later related to worm attrition, especially in the high dose group. However, it may also relate to decreased passage or increased destruction of eggs in the intestinal tissues. The marked decrease in eggs in the liver in the 2,000 dose group after 11 weeks PI suggests that pigs, like rhesus monkeys and baboons,<sup>24</sup> are able to destroy liver-deposited eggs fairly rapidly. No significant difference in the number of eggs in the liver per worm pair between the three groups was found at 4 and 11 weeks PI although this parameter tended to be higher for the 500 dose group at both time points. In mice infected with different doses of a People's Republic of China strain of *S. japonicum* for seven months,<sup>27</sup> eggs excreted in the feces and accumulating in the liver stabilized 12 weeks PI, while worm burdens remained constant and eggs deposited in the intestinal tissues increased throughout the study. A similar finding occurred with single worm pairs of a Philippine strain of *S. japonicum* transplanted into mice,<sup>31</sup> in which fecal and liver egg counts remained stable after 10 weeks PI, while intestinal egg counts increased greatly. The results of these studies suggest that in mice the reduction of eggs in the feces and liver over time may be due to factors other than reduced worm fecundity. Intestinal egg counts were not performed in our experiment so it is unknown whether the same phenomenon occurs in

pigs. However, worm death at later time periods was a feature of our results.

Extremely strong correlations were observed between fecal egg counts at the time of maximum excretion, liver egg counts, and worm burdens. This indicates that the passage of *S. japonicum* eggs in the feces of pigs during early patency is a good indicator not only of the intensity of infection but also of the number of eggs accumulating in the liver, and could therefore provide information important for mathematical modeling of the disease. This is in contrast to mice in which the number of eggs in feces at seven and 10 weeks PI correlated poorly with the number of eggs in the livers of mice infected with a Philippine strain of *S. japonicum*.<sup>31</sup>

The pigs in our study exhibited virtually no signs of illness except for diarrhea at the time of patency in a few pigs, and did not develop clinicopathologic abnormalities, other than blood eosinophilia, even in the highest dosed group. This is in contrast to rabbits infected with the Philippine strain and chimpanzees infected with the Japanese strain of *S. japonicum*, which developed anemia and hypoalbuminemia from moderate infections with the parasite.<sup>32,33</sup> The reason for the eosinophilia observed in all four groups late in the experiment is unknown. Destruction of adult schistosomes could have been a cause of the late eosinophilia but this would not have had any effect on the control pigs. Most of the schistosome-infected and control pigs were found to be excreting large numbers of *B. coli* cysts during the latter part of the experiment and this may have played a role in eosinophil levels.

The gross intestinal lesions found in the present experiment were similar to those described in pigs infected with a high single dose (5,000–6,000 cercariae) of *S. japonicum* for up to 26 weeks in the study by Yason and Novilla,<sup>30</sup> except that these investigators did not observe ulcerations. Intestinal lesions in schistosomiasis japonica in animals and humans tend to be focal or segmental<sup>11,24,34</sup> and sometimes ulcerated,<sup>31</sup> and the lesions of the pig seem to conform to this pattern.

In contrast to the marked generalized liver fibrosis in our heavily infected pigs, only slight hepatic fibrosis in portal and periportal areas was found in the study by Yason and Novilla.<sup>30</sup> In schistosomiasis there is generally a correlation between the severity of hepatic fibrosis and the number of eggs in the tissue<sup>35</sup> and the conflicting results may therefore be due to differences in worm distribution patterns and the number of eggs accumulating in the liver, data on which were not presented by Yason and Novilla.<sup>30</sup>

The typical hepatic lesion of chronic human schistosomiasis japonica, pipestem fibrosis, is characterized by extensive fibrosis around intrahepatic branches of the portal vein but only slight parenchymal destruction.<sup>36</sup> This lesion has been described also in *S. japonicum*-infected chimpanzees<sup>31</sup> and rabbits.<sup>37</sup> Gross lesions resembling pipestem fibrosis were not observed in our experiment. However, severe hepatic fibrosis similar to the pipestem type with a fibrous thickening around portal veins has been demonstrated in pigs 12 months after massive experimental exposure to another schistosome, *S. incognitum*.<sup>38</sup> This indicates that gross liver lesions more similar to those seen in chronic human schistosomiasis japonica may develop in the pig, but whether

those can be induced by *S. japonicum* remains to be elucidated.

Hepatic fibrosis was quite extensive in some pigs, but the decrease in severity with time in all dose groups indicates that these changes are reversible. The mechanism behind this in pigs is not known, but a persistent increase in collagenolysis concurrent with decreased collagen synthesis has been suggested in rabbits, in which *S. japonicum*-induced advanced hepatic fibrosis regressed after anthelmintic treatment or spontaneous reduction in egg output.<sup>39</sup>

In summary, *S. japonicum* worms were located primarily in the large intestinal vasculature of pigs. After several weeks of patency, pigs with high intensities of *S. japonicum* single infection were able to effectively eliminate the majority of adult worms while maintaining a stable population of immature schistosomes. This was shown by reduced viable worm pair numbers, liver and fecal egg counts, and pathologic lesions with increased duration in this dose group. The continual presence of immature worms six months after infection was a common feature for all dose groups. Strong correlations between worm burdens and egg counts from both the liver and feces during the acute phase of infection suggests that fecal egg counts of pigs during this period may be used to estimate not only the intensity of infection but also the extent of hepatic lesions.

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