RECOVERY AND DISTRIBUTION OF *ASCARIS SUUM* SUPERIMPOSED ON A *SCHISTOSOMA JAPONICUM* INFECTION IN PIGS

Anne B Helwigh and Henrik O Bøgh

Danish Centre for Experimental Parasitology, Royal Veterinary and Agricultural University, Ridebanevej 3, DK-1870 Frederiksberg C, Denmark

Abstract. The aim of this study was to examine the effect of a primary patent *Schistosoma japonicum* infection on the establishment and location of a superimposed *Ascaris suum* infection in pigs. The study comprised two experiments each containing two groups of pigs. In the first experiment, 7 pigs were injected intramuscular (im) with 800 *S. japonicum* cercariae and inoculated with 1,000 *A. suum* eggs 11 weeks post primary infection (ppi) and 8 pigs were inoculated with 1,000 *A. suum* eggs at the time of challenge infection. In the second experiment, 7 pigs were injected im with 1,100 *S. japonicum* cercariae and inoculated with 1,000 *A. suum* eggs 16 weeks ppi and 8 pigs were inoculated with 1,000 *A. suum* eggs at the time of challenge infection. All pigs were slaughtered 10 days after the *A. suum* challenge infection. The number of white spots caused by *A. suum* on the surface of the liver was significantly lower in the groups with primary infections of *S. japonicum* compared with the control groups. However, the present experiments did not demonstrate any effect of a primary *S. japonicum* infection on the total recovery and distribution of an *A. suum* challenge infection.

INTRODUCTION

Many epidemiological surveys have shown that concurrent parasite infections in man and domestic animals are common (Ajayi *et al,* 1988; Gbakima 1994; Ferreira *et al,* 1994; Boes *et al,* 1998). *Ascaris suum,* the large round worm in pigs, is especially common under extensive management systems and is found world wide (Jacobs and Dunn 1969; Roepstorff *et al,* 1992; Roepstorff and Nansen 1994; Boes *et al,* 1998). *Schistosoma japonicum* is a zoonotic trematode with a wide range of final hosts, including humans and the domestic pig (Kumar and de Burbure 1986; Boes *et al,* 1998). *S. japonicum* is highly prevalent in China, and the Philippines, where approximately two million people suffer from schistosomiasis caused by *S. japonicum* (Pittella 1997). *A. suum* and *S. japonicum* coexist in the endemic areas of *S. japonicum* (Boes *et al,* 1998) and both infections usually cause marked pathological reactions in the liver. *S. japonicum* eggs are trapped in the liver parenchyma and *A. suum* larvae cause lesions during their migration through the liver. Several investigations have shown that concurrent infections with *Schistosoma* spp and other pathogens may result in antagonistic or synergistic interactions influencing the disease pattern of the individual infections (see reviews by Chieffi, 1992 and Christensen *et al,* 1987). Thus, Chamone *et al* (1990) observed a negative correlation between egg counts of *Schistosoma mansoni* and *Ascaris lumbricoides* or *Trichuris trichiura* in humans, and Crandall *et al* (1966) observed a significantly lower number of *A. suum* larvae when *A. suum* was superimposed on a *S. mansoni* infection in mice. Correa-Oliveira *et al* (1988) demonstrated antibody cross-reaction against *S. mansoni* in sera from patients with *A. suum* or *Ancylostoma duodenale* living in an area nonendemic for *S. mansoni*. The focus of the present study was on the gross liver pathology in the host and the establishment and location of an *A. suum* infection superimposed on a primary *S. japonicum* infection in the pig.

Correspondence : Anne B Helwigh.
Fax: +45 35 282774; E-mail: abh@kvl.dk

MATERIALS AND METHODS

Experimental animals

Thirty helminth-naïve Danish Landrace/Yorkshire/Duroc cross-breed pigs were used in the present investigation. They were housed under helminth free conditions with free access to water. The pigs were fed twice daily with ground barley and protein supplements. Petkevicius *et al* (1995) demonstrated

that this feed provides optimal conditions for establishment and survival of intestinal helminth. At the time of the *A. suum* challenge infection the pigs in experiment 1 weighed 92 ± 8 kg and the pigs in experiment 2 weighed 100 ± 9 kg.

Parasite material

The *S. japonicum* isolate originates from the People's Republic of China and is maintained in *Oncomelania hupensis* at the Danish Bilharziasis Laboratory. Cercariae were obtained by shedding infected snails previously exposed to newly hatched miracidia. The miracidia is recovered from eggs in the livers of infected mice (Moloney and Webbe, 1982). The *A. suum* isolate was isolated in 1993 (Roepstorff and Murrell, 1997) and since maintained by repeated passage through helminth naïve pigs. The eggs were isolated from fresh feces and embryonated in H_2SO_4 in the dark at room temperature for at least 3 months before being used for infection. This embryonation procedure has been proved to result in highly viable eggs (Oksanen *et al*, 1990).

Experimental protocol

Thirty pigs were divided into two experiments each consisting of two groups of 7 or 8 pigs, respectively. The experimental outline is given in Table 1. The primary infections were given by intramuscular injection of Iscoves medium-suspended cercariae of *S. japonicum* (Willingham *et al*, 1996). Infective *A. suum* eggs were given as challenge infections by stomach tube 11 weeks post primary infection (ppi) in experiment 1 and 16 weeks ppi in experiment 2. The two different periods between primary infection and challenge infection were chosen on the basis of results obtained by Willingham *et al* (1998). Three fecal samples were taken from the pigs, one at the beginning of the experiment, one at challenge infection and one at slaughter in order to assure that no contamination from the environment had occurred .

Parasitological techniques

The concentration McMaster technic described by Roepstorff and Nansen (1998) was used in order to check for *A. suum* contamination. This method has a lower detection limit of 20 eggs per gram feces.

The animals were killed using a captive bolt pistol and exsanguinated. The liver, lungs and small intestine were removed immediately. The number of white spots on the surface of the liver caused by *A. suum* was determined. Only the compact white spots of the *A. suum* granulation-tissue type or lymphonodular type (Ronéus, 1966) larger than the typical *S. japonicum* granuloma (Warren *et al*, 1975) were counted. These spots ranged from 4-8 mm and were easily distinguished from the *S. japonicum*

Table 1

Experimental protocol. The primary infections were given by intramuscular injection of Iscoves medium-suspended cercariae of *Schistosoma japonicum*. The challenge infection with infective *Ascaris suum* eggs was given by stomach tube.

Exp no.	Group	No. of pigs	Primary infection	Challenge (weeks ppi)	Challenge infection	Slaughter (days pci)
1	A	7	800 Si	11	1,000 As	10
	B	8	-	11	1,000 As	10
2	A	7	1,100 Sj	16	1,000 As	10
	B	8	-	16	1,000 As	10

Sj = *S. japonicum*, *As* = *A. suum*, ppi = post-primary infection, pci = post challenge infection.

granulomas. Liver tissue egg counts from the *S. japonicum* primary infected pigs were determined following digestion of three 5 g samples from the left liver lobe (Bjrneboe and Frandsen, 1979; Bøgh *et al,* 1996). The right half of the liver and the entire lungs were cut into approximately 5 mm pieces using a kitchen blender. The larvae from the subsample of each liver (50% of the total volume) were isolated using the agar-gel method (Slotved *et al,* 1995) and larvae from a subsample of the lungs (25% of the total volume) were isolated using the macro Baermann technic (Eriksen *et al,* 1992). The small intestines were divided into four sections of equal length. The intestinal contents were embedded in agar to isolate small ascarids by the agar-gel method as described by Slotved *et al* (1997). All samples were fixed and stored in an iodine solution (6.25% iodine, 31.25% potassium iodine, 62.5% distilled water). Immediately before counting, the samples were decolorized with 3% thiosulphate solution.

Calculations and statistical analyses

All means were calculated as arithmetic means ± standard variation. The results from the subsamples of the liver and lungs were added up to 100%. Total *A. suum* worm burdens, number of larvae in the liver, lungs and four sections of the small intestine, and the number of white spot on the liver surface [log (y+1)] caused by *A. suum* were analysed using a two-factor analysis of variance to test for the effects of primary infection, period between primary and challenge infection, and their interaction. The proportion of the *A. suum* larvae in the liver, lungs and four sections of the small intestine were log (y+1) transformed and analysed by a repeated-measures analysis of variance to test for the effects of primary infection, period between primary and challenge infection and their interaction. Pearson's correlation coefficients were calculated to measure the correlations between *A. suum* worm burdens, *S. japonicum* eggs in the liver, and number of *A. suum*-induced white spots on the liver surface.

RESULTS

No clinical signs of disease were observed in any of the pigs, and all pigs had negative *A. suum* egg counts during the experiments. *A. suum* larvae were recovered from all pigs, and in all the *S. japonicum* infected pigs, *S. japonicum* eggs were recovered from the liver. At autopsy, *S. japonicum* worms were observed in the mesenteric veins around the intestine.

The mean number of white spots caused by *A. suum* on the liver surface is shown in Table 2. The majority of the spots were of the granulation-tissue type, but there were also a few spots of the lymphonodular type (Ronéus, 1966). In both experiments there was a significantly lower number of *A. suum* induced white spots on the surface of the livers in the group which was previously infected with *S. japonicum* (group A) compared to the

Table 2

Number of *Ascaris suum* induced white spots on the liver surface and recovery of *A. suum* larvae from the liver, lungs and four sections of the small intestine. For group designations see Table 1.

Exp No.	Group	White spots	Liver	Lung	SI	SI II	SI III	SI IV	Total
			No. of larvae						
1	A	15±24	0.6±1.4	141±84	111±59	88±63	70±65	39±63	449±102
	B	61±42	0	199±95	85±53	49±35	45±65	25±50	402±118
2	A	2±4	0	76±46	368±109	110±74	19±20	3±4	576±196
	B	49±34	0	43±32	373±159	149±71	37±28	3±2	604±192

SI=IV: Section I-IV of the small intestine.

challenge infected control group B ($p<0.0001$). In experiment 1, the number of *S. japonicum* eggs per gram (EPG) liver tissue was 100 ± 100 and there were fibrotic lesions in the livers with granulomas (< 4 mm) of the type typically observed in *S. japonicum* infections. In experiment 2, the *S. japonicum* EPG in liver tissue was 600 ± 482, and compared to experiment 1, more severe fibrotic changes and granulomas were observed.

The total recovery and relative location of *A. suum* larvae are shown in Table 2. In experiment 1 the recovery of *A. suum* was 449 for group A and 402 for group B. In experiment 2 the recovery of *A. suum* was 576 and 605 for groups A and B, respectively. There was no significant difference in the number of *A. suum* larvae recovered from groups A and B within each experiment ($p = 0.6$). However, there was a significantly higher number of *A. suum* larvae recovered from experiment 2, compared with experiment 1 ($p = 0.013$). The relative location of *A. suum* larvae in the liver, lungs and sections of the small intestine was not significantly different between the groups within each experiment. However, the relative location of *A. suum* larvae was significantly different between the two experiments ($p = 0.001$). Hence, 31 and 50% of the larvae were located in the lungs in groups A and B from experiment 1, while 7 and 12% of the larvae were located in the lungs in groups A and B from experiment 2. In experiment 2 the majority of the larvae were located in the 1st section of the small intestine, 65 and 62% for groups A and B, respectively. Whereas, only 25% and 21% of the larvae were located in 1st section of the small intestine of groups A and B in experiment 1. In both locations there were significant difference between experiments ($p < 0.008$).

Correlation between measures of infection

A Pearson correlation matrix was calculated for the following: *A. suum* worm burdens, *S. japonicum* eggs counts in the liver and *A. suum* induced white spots on the liver surface. No relevant correlations were observed for any of the groups.

DISCUSSION

In the present study the pigs infected with both *S. japonicum* and *A. suum* had more severe gross liver pathology with formation of small granulomas and tissue fibrosis compared to the pigs infected with *A. suum* only. The stronger liver pathology in the primary and challenge infected pigs is very likely due to the *S. japonicum* infection, since this parasite induces severe liver lesions in the pig (Willingham *et al,* 1998). Further, a significantly reduced number of large white spots (4-8 mm) caused by migrating *A. suum* was observed on the liver surface in pigs previously infected with *S. japonicum* compared with challenge control pigs. During a single infection with *S. japonicum* in pigs the eosinophil level is known to be elevated from week 6-16 (Willingham *et al,* 1997; 1998) and it was during this period the challenge infection in the present experiment was given. However, the present observations suggest that *S. japonicum* is either capable of suppressing the gross pathological reaction against *A. suum* or that it diverts the unspecific immune reaction in the liver from *A. suum* towards *S. japonicum.* However, the differences in liver pathology did not change the migration pattern of the *A. suum* larvae as there were no differences between the groups previously infected with *S. japonicum* and the control groups with respect to total recovery of larvae or the distribution of larvae in the liver, lung and different sections of the small intestine.

Unfortunately, it was not possible to infect the pigs in the two experiments with the same number of cercariae. The group receiving the highest number of cercaria (group A in experiment 2) had the highest number of eggs in the liver tissue. This group also had the most severe liver pathology. However, the different number of eggs trapped in the liver did not influence the migration and survival of *A. suum,* as there was no significant difference in the recovery of *A. suum* larvae between the two *S. japonicum* infected groups from the two experiments. Crandall *et al* (1966) challenge infected mice with 11,900 infective *A. suum* eggs 60 days after an infection with 100 *S. mansoni* cercariae and observed a reduction in the recovery of *A. suum* larvae. When the challenge infection was given 10 days after infection with *S. mansoni,* no difference was observed (Crandall *et al,* 1966). Their results were closely related with the degree of liver tissue damage of the mice and they suggested the resistance to *A. suum* to be induced by a non-specific tissue reaction (Crandall *et al,* 1966).

In the present study, a difference between the

two experiments was observed in the location of larvae. In experiment 1 a significant higher number of larvae was recovered from the lungs and significant lower number from the 1st section of the small intestine compared to experiment 2. This variability is not unusual for *A. suum* infections, *ie* other experiments at our laboratory, where pigs were infected 1,000 *A. suum* eggs and slaughtered 10 days after infection, have shown 80% of the larvae in the lungs and first section of the small intestine and more than 50% of the larvae in the first two section of the small intestine (Roepstorff *et al*, 1997).

It can be concluded that the gross liver pathology associated with an *A. suum* infection was less severe when a patent *S. japonicum* infection was established at the time of challenge infection. This might suggest that *S. japonicum* is either capable of inhibiting the host immune response against *A. suum* or diverts the unspecific immune reaction in the liver from *A. suum* towards *S. japonicum*. However, the further migration of the *A. suum* larvae was not affected as the recovery and distribution of larvae were similar to the control group.

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