SCHISTOSOMA JAPONICUM IN THE PIG: THE INFLUENCE OF AGE ON THE HOST/PARASITE RELATIONSHIP

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Abstract. The current study sought to elucidate a possible association between age and susceptibility to a primary infection with *Schistosoma japonicum* in pigs. Sixteen Landrace/Yorkshire crossbred specific pathogen-free pigs in three different age groups (group A-C), aged approximately 7, 24 and 37 weeks at the beginning of the experiment, were infected by intramuscular injections of 1,000, 1,500 or 2,400 cercariae, respectively. Fecal egg counts were obtained twice weekly from six to eight weeks post infection (wpi), and the pigs were killed 11 wpi. The number of worms collected were counted and sexed subsequent to perfusion. Tissue egg counts were estimated on samples from the liver. The worm recoveries for group A, B and C were 3.2%, 8.1% and 3.8%, respectively. No differences were observed between the male/female ratios of the three groups. The fecundity parameters, *ie*, fecal egg counts per mature female and liver egg counts per mature female, showed no significant differences between the three age groups. The results did not indicate any difference in susceptibility between the different age-groups of pigs to a primary infection with *S. japonicum*.

INTRODUCTION

Several investigations have demonstrated that the pig is a useful model for studying host/*Schistosoma japonicum* interactions, which for ethical reasons cannot be studied experimentally in humans (Willingham and Hurst, 1996). So far, no investigations regarding age-related susceptibility to *S. japonicum* in pigs have been reported. Recent investigations, however, have indicated that long term infected pigs may be able to regulate the worm population and thereby diminish the effect of the infection (Willingham *et al,* 1998a). It has also been reported that primary infected pigs were resistant to challenge infections with *S. japonicum,* and it was suggested that this resistance was due to an anti-worm effect (Willingham *et al,* 1997). Future experiments are planned to involve challenge or continuous infections of pigs with *S. japonicum*. Investigations into a possible age-related defence component is therefore warranted. In the present study we investigated the susceptibility of pigs of different ages to a primary infection with *S. japonicum*. It has previously been proposed by Gryseels (1994) that an age-dependent rise in resistance may be linked to sexual maturation of the host. Hence, the selected three age groups consisted of female pigs at weaning, pigs on the edge of sexual maturity and fully sexually mature pigs (Jacobsen *et al,* 1979). The experiment reported here sought to investigate and compare the chararacteristics of primary infections with *S. japonicum* in pigs of different ages.

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MATERIALS AND METHODS

Three age groups of female pigs, all specific pathogen-free crossbreds of Danish Landrace/Yorkshire/Duroc, were infected by intramuscular injection of *S. japonicum* cercariae as described by Willingham *et al* (1996). The three groups (A-C) were 7, 24 and 37 weeks old, respectively, at the time of infection. Each pig was infected with cercariae of an isolate of *S. japonicum* originating from the Anhui Province, People's Republic of China. The isolate is maintained in *Oncomelania hupensis* snails at the Danish Bilharziasis Laboratory. Table 1 outlines the experimental design. Group A (7 pigs) was infected with 1,000 cercariae, group B (5 pigs) with 1,500 cercariae and group C (4 pigs) with 2,400 cercariae. The pigs were housed, each age group together, but under similar and helminth free conditions, and fed a standard ration of ground barley and water *ad libitum*. Fecal samples were collected on day 42, 45, 49, 52, and 56 post infection. The number of schistosome eggs in the feces was determined by a combined filtration and sedimentation/centrifugation

technic (Willingham *et al,* 1998b). The pigs were killed and perfused 11 weeks post infection. One hour prior to perfusion, the pigs were orally administered 40 mg kg^{-1} praziquantel (Bayer, Germany) dissolved in propylene glycol using a plastic stomach tube. Thirty minutes prior to perfusion, the pigs were sedated with an intramuscular injection of 6 mg kg^{-1} azaperone (Sedaperone, Janssen, Belgium). When the pigs were sedated, 500 IU kg^{-1} heparin was administered through an intravenous catheder placed in an auricular vein. Subsequently, the pigs were killed by an overdose of pentobarbital (Mebumal). The perfusions of the portal systems were performed as described by Bøgh *et al* (1997). After the perfusions, the intestinal veins were examined manually for any residual worms. Tissue egg counts were conducted subsequent to digestion of 5 g samples from each of the left lateral, left medial, central, right medial and right lateral liver lobes of each pig (Bjørneboe and Frandsen, 1979; Bøgh *et al,* 1996).

One-way analysis of variance was used to test for differences between group means of male/female ratio, percentage establishment, fecal egg counts and tissue egg counts. Differences between the means of groups were considered significantly different at p-values < 0.05. The correlation between number of eggs deposited in the liver and the number of mature females was tested by Pearson correlation test.

Table 1
Experimental design: Number of pigs in each group, mean body weight at infection (kgs) and cercarial dose for each group.

Group	No. of pigs	Weight at infection (mean ± SD)	Cercarial dose
A	7	12.7 ± 1.3	1,000
B	5	89.2 ± 5.4	1,500
C	4	141.8 ± 9.4	2,400

RESULTS

At the time of perfusion, all pigs in the three age groups were found to be infected with *S. japonicum*. Except for a slight diarrhea in group A during the first few weeks of patency, no clinical signs of the infection were observed. However, all pigs in the study excreted cysts of the protozoan *Balantidium coli* throughout the fecal sampling period.

Table 2 summarizes worm establishment and the mean number of eggs per gram (epg) feces per mature female at the last sampling day and the mean number of eggs per gram liver tissue per female. The number of worms collected showed a high degree of variation within each group. The male/female ratio was found to be close to 1 with no significant differences between the three groups. The worm establishment varied from 3.2% in group A to 8.1% in group B with the differences between group A and B being significantly different. The 3.8% worm establishment in group C was not significantly different from either group A or B.

The mean fecal excretion peaked at day 49 post infection with 4.8 (SD 4.4) epg in group A, at day 52 [5.6 epg (SD 9.2)] in group B and at day 45 in group C [10.0 epg (SD 2.5)] with no significant differences between the three groups at any of the sampling days. No significant differences were seen in the fecal epg per mature female at the last sampling day.

The mean liver epg per mature female is shown in Table 2. No significant differences were found

Table 2
Mean number and standard deviation of total worms, establishment percentage, eggs per gram feces per mature female and eggs per gram liver tissue per mature female.

Group	Total no. of worms	Percentage establishment	Fecal-epg per female	Liver-epg per female
A	31.7 ± 21.9	3.2 ± 2.2 [a]	0.4 ± 0.5	15.3 ± 8.0
B	128.4 ± 53.5	8.1 ± 4.2 [b]	0.9 ± 1.4	19.6 ± 8.1
C	89.6 ± 21.6	3.8 ± 0.7 [c]	0.1 ± 0.1	11.4 ± 2.3

[b] > [a]: $p < 0.05$; [a] *vs* [c] and [b] *vs* [c]: not significant.
Fecal-epg per female and liver-epg per female: no significant differences between any groups.

between the groups. A strong correlation ($p < 0.001$) was observed when relating the number of eggs in the liver of all three age groups to the number of females found at perfusion.

DISCUSSION

The results observed in the present study indicates that age *per se* do not significantly influence the establishment and fecundity of *S. japonicum* in pigs.

Previous studies have shown that a major response in pigs to an infection with *S. japonicum* appears to be an anti-worm effect (Willingham *et al,* 1998a). It could therefore be expected that any age related decrease in susceptibility would affect the number of worms perfused from the mesenteric veins from the different age-groups. The results obtained from the present study showed no decrease in worm establishment with increasing age comparing pigs aged 7 and 37 weeks, respectively. The middle age-group had a higher establishment percentage than the youngest group, although in our opinion this difference can be contributed to the inherent variation in the pig model rather than any differences in susceptability between the groups. The recorded worm establishment percentages and variation are in line with previous published data for infection of pigs with *S. japonicum* (Willingham *et al,* 1997, 1998a; Johansen *et al,* 1997; Johansen 1998).

The obtained worm fecundity parameters (liver egg counts- and fecal egg counts per mature female) supported the conclusion that no differences in infection-characteristics occurred between the three age groups. No significant difference was found neither between the liver egg counts- nor fecal egg counts per mature female in the different groups. The highly significant correlation between liver egg counts and number of mature females also supported the conclusion that the characteristics of the primary infections in the three age-groups were not different. If pigs mounted an anti-fecundity response with increasing age it should be expected that the number of eggs per mature female would decrease with increasing age, and that the correlation therefore would be poor.

The different age groups of pigs received different cercarial doses. It could therefore be argued that a comparison between the three groups is confounded, since the different cercarial doses as well as age-related differences may affect parasitological parameters. From a previous study, however, this was shown not to be the case. Willingham *et al.* (1998a) infected three groups of 6-10 weeks old pigs with cercarial doses of 100, 500 and 2,000. Their study found no significant differences when percentage worm establishment, fecal EPG/worm pair and liver EPG/worm pair were compared between the groups. This suggest that a dose-related change in the infection characteristics did not occur. Thus, the comparison between the three age-groups receiving different doses in this study is valid.

In summary, the current study showed that the age of the pigs does not change the characteristics of a primary porcine infection with *S. japonicum* under the given circumstances. This observation is important for later continuous (trickle) or challenge infections. Indeed, it may rule out the possibility that any recorded change in response to a primary infection is due to maturation of the immune system or other age-related changes, for example the hormone status, of the experimentally infected pigs. However, the current study gives no indication whether the characteristics of challenge infections are different in pigs of different ages. Future studies into any age-related component of the characteristics of *S. japonicum* infections in pigs are therefore warranted.

ACKNOWLEDGEMENTS

The Danish National Research Foundation is thanked for providing financial support. The authors also wish to thank LE Petersen, NP Hansen, A Mehlsen, T Rasmussen and N Midtgaard for technical support. The pigs used in this study were treated in accordance with the Danish Animal Ethics Law.

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