

Experimental Infection of Danish Landrace/ Yorkshire Crossbred Pigs with *Schistosoma japonicum* from the People's Republic of China

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Willingham A. L., M. V. Johansen, B. J. Vennervald, N. Ø. Christensen and P. Nansen: Experimental infection of Danish Landrace/Yorkshire crossbred pigs with *Schistosoma japonicum* from the People's Republic of China. Acta vet. scand. 1994, 35, 395-400. – This study was undertaken to assess the suitability of Danish Landrace/Yorkshire (L/Y) crossbred pigs as experimental hosts of a Chinese mainland strain of *Schistosoma japonicum*. Pigs were exposed to 200, 500 or 1000 cercariae and parasite burdens were determined by perfusion after either 8 or 11 weeks. All pigs became infected with onset of faecal egg excretion 6 to 7 weeks following exposure to cercariae. The pattern of faecal egg excretion differed markedly among the individual animals. Gross hepatic pathological lesions of varying degrees were noted in all of the pigs. Schistosome worm recoveries ranged from 1.5 – 23.4% of the cercarial exposure dose. Most schistosome eggs recovered from the tissues, expressed as eggs/g tissue, were found in the rectum (91%), caecum (3.1%) and liver (5.1%). The results show that Danish L/Y pigs may serve as appropriate experimental final hosts of the Chinese mainland strain of *S. japonicum*.

Animal model; population biology; zoonosis.

Introduction

Schistosoma japonicum is a zoonotic Asian schistosome which parasitizes a wide range of mammalian hosts. Over a hundred million people are at risk of *S. japonicum* infection, which can cause life-threatening liver and colorectal disease (Mott 1982). Water buffaloes, cattle, pigs, dogs and rodents are considered important reservoir hosts, contributing to the transmission of *S. japonicum* (Basch 1986, Kumar & de Burbure 1986, He 1993, Xu et al. 1993).

Considering the anatomical, physiological and immunological similarities of man and pig (Swindle 1984, Phillips & Tumbleson 1986), porcine schistosomiasis japonica may be a relevant model for human *S. japonicum* infection. However, little is known about natural *S.*

japonicum infection in pigs and only few experimental studies have been conducted. Yason & Novilla (1984) were able to infect different breeds of pigs with a Philippine strain of *S. japonicum* and obtained some initial information on clinical, gross pathological and histopathological features of the infection. However, Reid & von Lichtenberg (1977) found that miniature pigs were unsuitable hosts for a Japanese strain of *S. japonicum*. Normal sized Chinese pigs investigated in a vaccine trial involving a Chinese mainland strain of *S. japonicum* were found to be suitable hosts, although the worm establishment was low (Shi et al. 1993). Further studies are needed to improve our understanding of the host-parasite relationship and the population

Table 1. *Schistosoma japonicum* infection of Danish Landrace/Yorkshire crossbred pigs. Details of experimental design and worm recovery from perfusions at 8 or 11 weeks following exposure to 200, 500 or 1000 cercariae/pig.

Pig	Cercarial dose	Duration of experiment (weeks)	Prepatent period	Schistosome recovery				
				Number of males/females /immatures	Total number of worms	Number of worm pairs	Worm recovery percentage	Male/Female ratio
1	1000	11	7	9/16/0	25	9	2.5	0.6
2	1000	11	7	6/9/0	15	6	1.5	0.7
3	1000	11	7	26/36/0	62	26	6.2	0.7
4	500	11	7	42/21/0	63	21	12.6	2.0
5	500	11	6	92/25/0	117	25	23.4	3.7
6	200	8	6	2/2/4	8	2	4.0	1.0
7	200	8	7	7/6/2	15	6	7.5	1.2
8	200	8	7	7/7/2	15	7	7.5	1.0
9	200	8	7	8/1/0	9	1	4.5	8.0
10	200	8	6	2/1/4	7	1	3.5	2.0

Table 2. Number and distribution of *Schistosoma japonicum* tissue eggs (eggs/g tissue, % distribution) in Danish Landrace/Yorkshire crossbred pigs 11 weeks following exposure to either 500 or 1000 cercariae.

Pig ¹	Schistosome egg counts/g tissue						
	Lung	Liver	Duodenum	Jejunum	Caecum	Colon	Rectum
1	0	250	117	17	17	0	19600
2	17	500	33	67	483	17	83
3	0	483	0	17	550	0	3100
4	0	917	33	100	467	0	22583
5	0	483	0	0	50	0	1033
Mean # eggs/g	3	527	37	40	313	3	9280
% of total	0.03	5.1	0.37	0.37	3.1	0.03	91.0

1 Pig numbers refer to pigs 1-5 listed in Table 1.

Table 3. Number of *Schistosoma japonicum* worm pairs recovered from Danish Landrace/Yorkshire pigs 11 weeks post-infection and various measurements of worm fecundity/worm pair.

Pig ¹	Number of worm pairs	Eggs/g rectal tissue/worm pair	Eggs/g liver tissue/worm pair	Eggs/g faeces/worm pair (at maximum excretion)
1	9	2178	28	10
2	6	14	83	16
3	26	120	81	10
4	21	1075	44	13
5	25	413	19	2
Mean	17	760	51	10

1 Pig numbers refer to pigs 1-5 listed in Table 1.

biology of *S. japonicum* in the porcine host. The present study was undertaken to assess the suitability of Danish Landrace/Yorkshire crossbred pigs as experimental hosts for a Chinese mainland strain of *S. japonicum*.

Materials and methods

Ten male Landrace/Yorkshire crossbred piglets, initially 8-12 weeks of age and weighing 10-15 kg, were used. The pigs were reared and housed indoors under specific pathogen-free conditions and were free from gastro-intestinal nematode infections. However, all pigs harboured infections with *Balantidium coli* throughout the experimental period.

A Chinese mainland strain of *S. japonicum* was used. The exposure to cercariae took place using the leg immersion technique with an exposure period of 30 min (van Wyk *et al.* 1975). Pigs were exposed to either 200 (n=5), 500 (n=2) or 1000 (n=3) cercariae each, and examination of faeces from week 5 onwards for eggs of *S. japonicum* was done using the modified Bell filtration method (Monrad *et al.* 1991). The pigs were euthanized and blood perfusion was performed after either 8 or 11 weeks using a modification of the technique described by Smithers & Terry (1965). Schistosomes recovered were sexed and counted. Gross pathological lesions were noted at the time of perfusion, and 2 g samples were taken from the liver, duodenum, jejunum, caecum, colon, rectum and lung, respectively, for determination of tissue schistosome egg counts using the potassium hydroxide tissue digestion technique described by Bjørneboe & Frandsen (1979).

Results

Infections with *S. japonicum* were established in all pigs, with faecal egg excretion starting week 6 after infection in 3 pigs and week 7 in the remaining 7 pigs (Table 1). Infections had a sub-clinical course in all animals. Faecal egg excretion

in pigs followed beyond week 8 (Fig. 1) increased steadily until week 10 in all pigs. In 3 of 5 pigs, faecal egg excretion at week 11 remained comparable with that at week 10, whereas in the remaining 2 pigs faecal egg excretion increased markedly from week 10 to week 11. The highest value of 272 eggs/g faeces was observed week 11 in pig #4 exposed to 500 cercariae (Fig. 1).

S. japonicum worm establishment varied greatly among the pigs, with recovery percentages ranging from 1.5% to 23.4% of the cercarial exposure dose (Table 1). In addition, the adult male:female worm ratio was quite variable, ranging from 8 to 0.6.

Gross pathological lesions of varying degrees were seen in livers of all pigs at the time of perfusion. The lesions appeared as diffuse, white, pinpoint areas on the liver surface characteristic of schistosome egg granulomas (Fig. 2 & 3). These lesions were seen to coalesce in the more seriously affected livers giving them a greyish-white appearance. Macroscopic lesions of the intestinal tract were not observed. As appears in Table 2, most tissue-deposited *S. japonicum* eggs, as expressed by mean # eggs/g tissue, were found in the rectum (91%), with a moderate number of eggs also found in the liver (5.1%) and caecum (3.1%). Very few eggs (<1%) were found in the duodenum, jejunum, colon and lungs, respectively. The tissue egg distribution pattern, as well as the total tissue egg counts/pig, differed markedly between pigs.

Concerning fecundity measures, the number of eggs/g rectal tissue/worm pair was quite variable, being in the range of 14 to 2178 eggs/worm pair in the different pigs (Table 3). The number of eggs/g liver tissue/worm pair was less variable, being in the range of 19 to 83 eggs/worm pair. Faecal egg excretion (expressed as eggs/g faeces/worm pair) at the time of maximum excretion was comparable among 4 of the 5 pigs followed, with the figures ranging from 10 to 16 eggs/g faeces/worm pair.

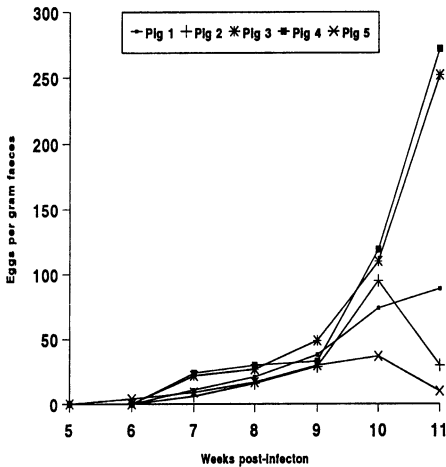


Figure 1. Number of *Schistosoma japonicum* eggs excreted per g faeces of individual Danish Landrace/Yorkshire crossbred pigs infected with 500 or 1000 cercariae during weeks 5 to 11 post-infection. (Note: Pig numbers refer to pigs 1-5 listed in Table 1).

Discussion

The present study shows that Danish Landrace/Yorkshire crossbred pigs are suitable experimental final hosts for the Chinese mainland strain of *S. japonicum* investigated. All pigs became infected, eggs were passed in their faeces and gross pathological lesions were noted in all of their livers. The length of the prepatent period, i.e. 6-7 weeks, exceeded that previously reported for the Philippine strain of *S. japonicum*, i.e. 4-5 weeks, and the worm recovery rate of 1.5 to 23.4% in the present study was in the lower range of the 18.9 to 30% recovery rate reported for the Philippine strain in pigs (Yason & Novilla, 1984). In the latter study, the coverslip method of percutaneous exposure was used. The variation in percentage worm establishment in the present study may be due to variations between pigs in susceptibility and response to *S. japonicum* infection, but variations in the biological characteristics of

different batches of cercariae may also play a role. It is possible that the *S. japonicum* worm establishment may increase with less variability using other infection procedures such as the coverslip percutaneous method or various larva injection methods (James & Taylor, 1976, Moloney & Webbe, 1982, Yason & Novilla, 1984). However, even following optimal infection procedures, a certain variation in worm establishment is to be anticipated.

Variations in total tissue egg counts, tissue egg counts per worm pair and the relative distribution of eggs of *S. japonicum* among different tissues were recorded. These variations may reflect differences in a given *S. japonicum*/pig relationship which can be affected by a range of different and sometimes interacting factors. However, the results leave no doubt that *S. japonicum* infection in pigs mainly affects the rectum and liver. This is similar to the human host (Mott 1982, Chen 1991), and supports the appropriateness of the pig as a model host for human schistosomiasis japonica.

Eggs of *S. japonicum* are deposited in clusters in vessels surrounding the intestinal tract where the schistosomes are located, whereas those in the liver are usually a result of embolization of eggs originally deposited in the intestinal vessels (Cheever & Duvall, 1982). This needs to be considered in assessment of numbers and patterns of distribution of tissue deposited eggs.

The results show that the use of porcine schistosomiasis as a model for human schistosomiasis japonica has great merits. Further studies on the porcine schistosomiasis japonica model will address population biological and dynamical aspects of infection under the influence of variables such as exposure dose, number of exposures, duration of infection, and age and nutritional status of the pig host. Immunological, clinicopathological and histopathological studies will be added to ensure a full elucidation of central aspects of the *S. japonicum*/pig model.



Figure 2. Liver of a Danish Landrace/Yorkshire crossbred pig 8 weeks after infection with 200 cercariae of *Schistosoma japonicum*.



Figure 3. Liver of a Danish Landrace/Yorkshire crossbred pig 11 weeks after infection with 1000 cercariae of *Schistosoma japonicum*.

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References

- Basch PF*: Schistosomiasis in China: An update. *Amer. J. Chi. Med.* 1986, *14*, 17-25.
- Bjørneboe A, Frandsen F*: A comparison of the characteristics of two strains of *Schistosoma intercalatum* Fisher, 1934 in mice. *J. Helminthol.* 1979, *53*, 195-203.
- Cheever AW, Duvall RH*: *Schistosoma japonicum*: Migration of adult worm pairs within the mesenteric veins of mice. *Trans. R. Soc. Trop. Med. Hyg.* 1982, *76*, 641-645.
- Chen MG*: Relative distribution of *Schistosoma japonicum* eggs in the intestine of man: A subject of inconsistency. *Acta Tropica* 1991, *48*, 163-171.
- He YX*: Biology of *Schistosoma japonicum*. *Chi. Med. J.* 1993, *106*, 576-583.
- James ER, Taylor MG*: Transformation of cercariae to schistosomula: A quantitative comparison of transformation techniques and of infectivity by different injection routes of the organisms produced. *J. Helminth.* 1976, *50*, 223-233.
- Kumar V, de Burbure G*: Schistosomes of Animals and Man in Asia. *Helminth. Abstr.* 1986, *55*, 469-480.
- Moloney NA, Webbe G*: A rapid method for the infection of laboratory mice with *Schistosoma japonicum*. *Trans. R. Soc. Trop. Med. Hyg.* 1982, *76*, 200-203.
- Monrad J, Christensen NØ, Nansen P*: Acquired resistance in goats following a single primary *Schistosoma bovis* infection. *Acta Tropica* 1991, *48*, 69-77.
- Mott KE*: *S. japonicum* and *S. japonicum* like infections. In: *Schistosomiasis: Epidemiology, treatment and control.* (Jordan P and Webbe G, eds.). William Heinemann Medical Books Ltd., London 1982, pp. 128-149.
- Phillips RW, Tumbleson ME*: Models. In: *Swine in Biomedical Research* (Tumbleson ME, ed) Plenum Press, New York 1986, pp. 437-440.
- Reid WA, von Lichtenberg F*: Experimental *Schistosoma japonicum* infection in miniature pigs. *J. Parasitology* 1977, *63*, 392-394.
- Shi YE, Jiang CF, Han JJ, Li YL, Ruppel A*: Immunization of pigs against infection with *Schistosoma japonicum* using ultraviolet-attenuated cercariae. *Parasitology* 1993, *106*, 459-462.
- Smithers SR, Terry RJ*: The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of the adult worms. *Parasitology* 1965, *55*, 695-700.
- Swindle MM*: Swine as a replacement for dogs in surgical teaching and research laboratories. *Lab. An. Sci.* 1984, *34*, 383-385.
- van Wyk JA, Heitmann LP, van Rensburg LJ*: Studies on schistosomiasis. 7. A comparison of various methods for the infestation of sheep with *Schistosoma mattheei*. *Onderstepoort J. Vet. Res.* 1975, *42*, 72-74.
- Xu ST, Shi FH, Shen W, Lin JJ, Wang YH, Lin BF, Qian CG, Ye P, Fu LC, Shi YJ, Wu WJ, Zhang ZD, Zhu HJ, Guo WG*: Vaccination of bovines against schistosomiasis japonica with cryopreserved-irradiated and freeze-thaw schistosomula. *Vet. Parasitology* 1993, *47*, 37-50.
- Yason CV, Novilla MN*: Clinical and pathologic features of experimental *Schistosoma japonicum* infection in pigs. *Vet. Parasitology* 1984, *17*, 47-64.

Sammendrag

Eksperimentel infektion af Dansk Landrace/Yorkshire grise med Schistosoma japonicum fra den Kinesiske Folkerepublik.

Egnetheden af Dansk Landrace/Yorkshire (L/Y) grise som eksperimentelle værter for et isolat af *Schistosoma japonicum* fra den Kinesiske Folkerepublik blev undersøgt. Grise eksponeredes for 200, 500 eller 1000 cercarier, og bestemmelse af ormebyrdens størrelse fandt sted ved perfusion 8 eller 11 uger efter infektion. Alle grise inficeredes med fækal ægudskillelse startende 6 til 7 uger efter infektion. Det fækale ægudskillelsesmønster varierede markant mellem grisene. Makroskopiske, lever-patologiske læsioner af forskellig grad sås hos alle grise. Schistosomorme etableringen varierede mellem 1,5% og 23,4% af anvendte cercarier. De fleste schistosomæg, udtrykt som æg/gram væv, fandtes i rektum (91%), med mindre forekomster i caecum (3,1%) og lever (5,1%). Undersøgelsen viste at danske L/Y grise kan fungere som velegnede eksperimentelle slutværter for det anvendte isolat af *S. japonicum*.

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