Congenital transmission of *Schistosoma japonicum* in the rabbit

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Abstract

Fourteen pregnant rabbits were each infected with 300 cercariae of *Schistosoma japonicum* and divided into two groups. Group M ($n=8$) was infected during mid-gestation (the organogenetic stage) and group L ($n=6$) was infected during late-gestation (the post-organogenetic stage). Mother rabbits and rabbit kittens were killed 45–60 days after infection and perfused in order to obtain worm counts. Furthermore, faecal egg counts and tissue egg counts from livers were obtained from the mother rabbits as well as the rabbit kittens. All mother rabbits became infected harbouring 207.6 ± 20.2 and 220.0 ± 27.5 adult worms in group M and L, respectively. In groups M and L, 13.5% and 46.7% of the kittens were infected, respectively. In 12 of 14 litters at least one kitten was infected. The infected kittens harboured between one and three adult *S. japonicum*. The livers of the kittens infected with a worm pair displaced lesions as a result of egg deposition. The results, therefore, show that congenital transmission of *S. japonicum* can occur in rabbits. The close anatomical resemblance between the rabbit and human placenta may be indicative of the presence of congenital transmission of *S. japonicum* infection in humans.

Introduction

Schistosomiasis caused by the zoonotic blood fluke *Schistosoma japonicum* is solely observed in the Far East including China, the Philippines and Indonesia where an estimated two million people are infected (Kumar & Burbure, 1986; McGarvey *et al*. 1999). More than forty mammals, including the rabbit, act as natural definitive hosts (Kumar & Burbure, 1986; Chen, 1993). Normally, *S. japonicum* cercariae infect the definitive hosts through the skin during contact with water. Early and recent investigations, however, suggest the possible existence of transplacental transmission from mother to offspring (Narabayashi, 1914, 1916; Kikuchi, 1957; Wang, 1958; Okabe, 1961; Willingham *et al*., 1999). Thus, Narabayashi (1914) recovered schistosomes from rabbits born by experimentally infected mothers. Furthermore, Willingham *et al*. (1999) observed that piglets born by experimentally infected sows during mid-to-late pregnancy were all infected with *S. japonicum*. The importance of this alternative route of transmission is obvious not only in animals but also in humans and clearly further investigations are needed. The rabbit placenta exhibits a haemomonochorial placenta composed of three layers, an anatomical feature resembling the placenta anatomy in humans (Björkman *et al*., 1989; Wooding & Flint, 1994). Therefore, the rabbit is a natural choice of host model when considering possible implications of *S. japonicum* infections in pregnant women.

The purpose of the present investigation was to

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Table 1. Worm burdens, faecal egg counts (FEC) and tissue egg counts (TEC) of mother rabbits and rabbit kittens. Mother rabbits were infected with 300 cercariae of *Schistosoma japonicum* after 14–16 days of gestation (group M).

Mother rabbit	Gestation day of infection	Infection period (days) in mother rabbits	Litter size	No. infected rabbit kittens	No. worms recovered from mother rabbit	No. worms recovered from rabbit kittens	Mean liver TEC from rabbit kittens	Mean FEC from rabbit kittens
1	16	57	8	1	218	1	0	0
2	16	57	6	0	233	0	0	0
3	15	57	8	0	195	0	0	0
4	14	60	7	1	211	1	0	0
5	14	60	6	1	nd	2	0	0
6	15	60	6	2	181	1–2	623.3	60.0
7	14	60	4	1	nd	1	0	0
8	14	60	7	1	nd	2	170.0	10.0
Mean			6.5 ± 1.3		207.6 ± 20.2		397 ± 320	35 ± 35

nd, Not done.

confirm early observations on congenital transmission of *S. japonicum* in rabbits and to undertake a further parasitological investigation of the infection. Furthermore, the transmission of *S. japonicum* to offspring from mother animals infected in the middle of the gestation was compared with transmission patterns when infections were given to mother animals during late gestation.

Materials and methods

Fourteen pregnant rabbits of the New Zealand breed were percutaneously infected with 300 *S. japonicum* cercariae using a slightly modified coverslip method described by Yason & Novilla (1984). The parasite material originated from a field isolate obtained from *Oncomelania hupensis* snails in the Zhejiang Province of the People's Republic of China. The life cycle has since been maintained in the laboratory at the Institute of Parasitic Diseases, Zhejiang Academy of Medical Sciences. Eight mother rabbits (Group M) were infected 14–16 days after gestation whereas six mother rabbits (group L) were infected 21–26 days after gestation. These infection times correspond to the organogenetic stage of embryonation, i.e. day 6–18 of gestation (group M) and the foetal developmental stage (group L) (Jørgensen, 1998). The rabbits were conventionally housed in cages at the Institute of Parasitic Diseases, Zhejiang Academy of Medical Sciences and given standard feed and water *ad libitum*. Mother rabbits as well as the rabbit kittens were killed 46–60 days after infection and worm counts were obtained after perfusion of the mesenteric veins. The number of eggs in the livers was determined using a modification of the technique described by Bjørneboe & Frandsen (1979). Faecal egg counts were obtained by counting all eggs from 1 g of faeces subsequent to cleaning through a 140 μm mesh sieve. One-way analysis of variance was used to test for differences in litter size and number of worms recovered from mother rabbits.

Results

The gestation period lasted the normal 30 days for rabbits. Adult worms of *S. japonicum* were found in five of eight mother rabbits in group M (table 1). However, three mother rabbits in this group died from clinical acute schistosomiasis 60 days after infection whereby worm counts could not be obtained. Infected rabbit kittens were, however, found in all three litters of these mother animals indicating that the mother animals also had been infected. All mother rabbits of group L harboured adult worms (table 2). The number of adult worms in mother rabbits did not differ significantly between the two groups. The mean litter size of group M was 6.5 ± 1.3 and did not differ significantly from group L (5.0 ± 1.9).

In group M, 52 rabbit kittens from eight mother rabbits were born 14–16 days after infection. Adult *S. japonicum* worms were observed in six of the eight litters. However, only seven of the 52 rabbit kittens (13.5%) were infected

Table 2. Worm burdens, faecal egg counts (FEC) and tissue egg counts (TEC) of mother rabbits and rabbit kittens. Mother rabbits were infected with 300 cercariae of *Schistosoma japonicum* after 21–26 days of gestation (group L).

Mother rabbit	Gestation day of infection	Infection period (days) in mother rabbits	Litter size	No. infected rabbit kittens	No. worms recovered from mother rabbit	No. worms recovered from rabbit kittens	Mean liver TEC from rabbit kittens	Mean FEC from rabbit kittens
1	26	46	5	2	211	1–3	0	0
2	23	49	5	2	252	3	nd	nd
3	25	48	5	1	251	2	190.0	43.3
4	21	48	8	4	185	1–3	0	0
5	23	50	5	4	198	1–3	216.7	23.3
6	25	48	2	1	223	1	0	0
Mean			5.0 ± 1.9		220.0 ± 27.5		203 ± 19	33 ± 14

nd, Not done.

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at slaughter. Five of these seven rabbit kittens (71.4%) harboured only male worms. A similar observation was made in group L where ten of 14 infected rabbit kittens (71.4%) harboured male worms only. In group L, 14 of 30 rabbit kittens (46.7%) were infected at slaughter and adult *S. japonicum* worms were found in all six litters in this group. Six rabbit kittens in group M and in group L harboured worm pairs. Comparable tissue egg counts as well as faecal egg counts were obtained (tables 1 and 2).

Based on subjective observations, no gross morphological and size differences were detected between the worms obtained from the mother rabbits and the kittens. However, the livers of rabbit kittens infected with a worm pair displaced lesions as a result of egg deposition. These kittens appeared weaker and less vigorous when compared with uninfected rabbit kittens or in those with a single sex infection of *S. japonicum*.

Discussion

Percutaneous penetration of infective *S. japonicum* cercariae has long been recognized to be the main route of infection. However, although some early accounts of the possible existence of congenital transmission of infection were proposed (Narabayashi, 1914; Kikuchi, 1957; Wang, 1958; Okabe, 1961), it was not until the study of Willingham *et al*. (1999) that the subject of congenital transmission was investigated further. These authors showed that congenital transmission of *S. japonicum* can occur in pigs during the latter half of pregnancy. Thus, worms were recovered from all 26 piglets born by three sows infected during the 10th week of gestation. The worm burdens in the piglets ranged from two to 71. Our experimental findings showed that congenital transmission of an *S. japonicum* infection can occur in rabbits.

The infection time in our study influenced the establishment rate in the rabbit kittens as the late infection time resulted in significantly more worms recovered from the rabbit kittens compared with the early infection time. However, Willingham *et al*. (1999) observed a more pronounced difference between piglets born by sows infected in early pregnancy (4 weeks gestation) and piglets born by sows infected in later pregnancy (10 weeks gestation). Thus, none of the piglets from the former group were infected whereas all in the latter group were infected. The differences in the rate of transmission between the time of infection correlate with the organogenetic and the post-organogenetic stages of the two animals species (Jørgensen, 1998). There may therefore be anatomical and physiological reasons why the congenital transmission is more pronounced in the later stages of foetal development in both species. It is believed that the schistosomulae are spread hematogenically and that the foetus becomes infected via the placenta after the initial schistosomulum stage in the lungs of the mother rabbits.

Several studies have now been undertaken using the pig as a model for *S. japonicum* in humans (e.g. Willingham & Hurst, 1996; Johansen *et al.*, 1997; Willingham *et al.*, 1997, 1998). The close resemblance between the human and rabbit placenta anatomy may, however, make the rabbit a more suitable model when specifically studying congenital transmission of this parasite. Thus, the morphogenesis of human and rabbit foetal membranes indicates a very close affinity, both belonging to a group of animals exhibiting a haemochorial placenta (Björkman *et al.*, 1989). On the other hand, the pig belongs to the epitheliochorial group where two maternal endothelial and epithelial layers are present.

In 1916, Narabayashi observed *S. japonicum* eggs in the faecal matter of new-born children with no history of water contact. This is the first evidence for congenital transmission of schistosomiasis japonica in humans. Furthermore, a recent parasitological survey of children aged 0–4 years showed that between 1.8% and 9.3% were infected in four provinces of lake regions and Sichuan and Yunnan provinces of China (MOHP, 1992; Xie *et al*., 1996). However, more closely controlled studies are warranted in order to verify the existence of congenital transmission in humans. Considering the higher prevalence (18.8% versus 13.7%) in women in the endemic region of Yunnan province of China and that women are the main labour force in the rice fields (Tang *et al*., 1997) more emphasis should be put into investigations on congenital transmission of schistosomiasis japonica in humans. Not least the effects of treatment on mothers and offspring and reasons for stillbirth occasions should be investigated in relation to humans. Such studies may result in warnings against pregnant women having contact with infested water, e.g. rice fields. Water buffaloes and cattle are considered to be the main reservoir hosts of *S. japonicum* in mainland China (McGarvey *et al*., 1999). These animals are used extensively in the rice fields in China. Therefore, disease control of these animals is highly needed.

In Zimbabwe, 299 pregnant women were examined for infection with schistosomes. The frequency of stillbirth or infant mortality was 16% among mothers who previously had schistosome infection (*S. mansoni* and *S. haematobium*) compared with 8% among those who had no history of schistosome infection (Patana *et al*., 1995). This, however, is not a positive indication of the existence of congenital transmission of African human schistosomiasis and further controlled studies are needed.

In conclusion, we have confirmed the early finding by Narabayashi (1914) and provided evidence for congenital transmission of a *S. japonicum* infection in rabbits. The role of human congenital transmission of schistosomiasis japonica clearly needs to be further investigated and attempts to pursue this are being taken.

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