



Several Immunological Parameters in Rabbit Kittens Born to *S. japonicum* - infected Mothers

SALIM Abdel-Moneim Mohamed^{1*}, ABDELLA Mutaz Ahmed²

¹Department of Medical Microbiology, Faculty of Applied Medical Sciences, Taif University, Taif 21974, Saudi Arabia.

²Department of Biotechnology, Faculty of Science and Education, Taif University, Taif 21974, Saudi Arabia.

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*Corresponding Author

Abdel-Moneim Mohamed Salim

E-mail: salimabdelmoneim55@hotmail.com

@hotmail.com

Phone: 966-506746672,

Fax: 966-02-7274290

ABSTRACT

In this study we explored the rabbit as an animal model for the congenital infection of *schistosomiasis japonica* and assessed the effect of a congenital *S. japonicum* infection on the resistance of rabbit kittens to a postnatal challenge infection.

Kittens were challenged 17-19 weeks after the primary infection of their mothers. Perfusion was undertaken six weeks after the challenge. At this time parasitological, pathological and immunological parameters, worm reduction rate, granuloma size reduction rate, egg reduction rate, IgG and IgM responses were assessed and compared to that of kittens born to un-infected mothers.

The overall prevalence of congenital infection in kittens of infected mothers was 20% (12/60). After a postnatal challenge infection, prenatally infected kittens had a 54.66% worm reduction rate, 41.45% egg reduction rate, and 51.76% granuloma size reduction rate compared to naive kittens. Congenital infection decreases the IgM responses by 39.47 % while it increases the IgG responses by 56.22 %.

Together, these results indicate that congenital infection induce long-term effects on pathology and immune response patterns in rabbits subsequently challenge with *S. japonicum* cercariae.

Keywords:

Congenital infection, Rabbits, *S. japonicum*

BACKGROUND

Schistosomiasis japonica, the causative organism for schistosomiasis, is solely prevalent in Southeast Asia where an estimated two million people are infected and 69–75 million individuals at risk of infection. It is unique among the major schistosomes infecting humans, as zoonotic transmission is important, with domesticated and wild animals serving as reservoir hosts of the parasite, and an amphibious snail as intermediate host. Although human infection and disease caused by *S. japonicum* have been reduced in China and Philippines, further reductions may be difficult because of the continual transmission from infected animals.

Normally, humans become infected with *Schistosoma japonicum* when cercarial forms of the parasite—released by freshwater snails—penetrate the skin during contact with infested water. When active or acute infections coincide with pregnancy, the conditions also exist for the possible transplacental passage of the parasite (schistosomulae) to the developing fetus. This mode of schistosome transmission is known as vertical transmission that leading to congenital infection of the fetus and has been reported by many researchers in a wide range of animals such as mice, rabbits, guinea pigs, dogs, goats, water buffaloes and cattle (Narabayshi, 1914; Kikushi, 1957; Wang, 1958; Okabe, 1961; Willingham et al., 1999; Qian et al., 2000).

Immune relations between mothers and children have been demonstrated or detected in many diseases found in human. Many studies on different human parasitic diseases hypothesized that infection status of the mother may determine whether or not individuals who later become infected develop severe disease. Hightower et al. (1993) showed that a child born to a microfilaremic mother is 2.9 times more likely to become microfilaremic than a child born to an amicrofilaremic mother. The same pattern is found in malaria-endemic areas where non-endemic individuals react differently than indigenous people (Rasheed et al., 1995).

Studies of animal models infected with schistosome showed that their offspring have altered immunity to subsequent infection (Lewert and Mandlowitz, 1969). Epidemiological evidences, combined with animal experiments, indicate that the pattern of disease and pathology in human schistosomiasis relates to previous exposure and immunologic experience to the parasite. Thus, most children born in areas endemic for schistosomiasis may be born of infected mothers. Individuals who move from non-endemic to endemic areas often experience more severe acute disease and subsequent pathology than indigenous populations (Morieatry and Lewert, 1985; Nash et al., 1982).

Intensity and duration of the infection are major determinants, but other factors are also involved. These include genetic background of the host, nutritional status, parasite strain differences, and frequency of

infection. Maternal infection status has been proposed by many authors in order to explain the individual differences (Butterworth and Thomas 1999; Pearce and Andrew, 2002).

The aim of the present study was to assess the effect of a congenital *S. japonicum* infection on the resistance of rabbit kittens to a postnatal challenge infection. We assessed several immunological parameters in kittens born to *Schistosoma*-infected mothers in comparison to those born to non-infected ones.

2. MATERIALS AND METHODS

2.1. Experimental Animals

The experimental hosts were New Zealand white rabbits aged 16–17 weeks at the beginning of the experiment. The animals were allocated into three groups:

Group A (n=4): animals in this group had IgM⁺ antibody titers and their kittens represent the congenital +challenge group.

Group B (n=2): animals in this group had no serological evidence for infection with *S. japonicum*, their kittens represent the exposed +challenge group.

Group C (n= 3): their kittens represent the challenge control group.

2.2. Challenge Infection

18–20 weeks after infection of their mothers, kittens were challenged individually with a single dose of 100 *S. japonicum* cercariae (Chinese strain) through a shaved abdominal skin using the cover slip method described by Peters and Warren (1969) and slightly modified by Qian et al. (2000). Rabbits will subsequently bred and maintained in isolators at the University of Huazhong animal facility, allowed free access of water and fed commercial rabbit chow.

2.3. Antigens:

SEA and AWA *Schistosoma japonicum* were kindly provided by Head Department of Pathogenic Biology, Huazhong University, China. Antigen to be used in ELISA was a mixture of 20 µl of SEA (at 1:500 dilution) and 300 µl of AWA (at 1:1500 dilution) and 30 ml of carbonate–bicarbonate buffer (Ph 9.6).

2.4. Worm Recovery

Adult worm were enumerated by hepatic perfusion of rabbits 6 weeks after challenge infection. The parasites harvested from each animal were counted.

2.5 Tissue Egg Counts (EPG):

At the time of perfusion, in all rabbits, one piece of liver (about 5 g) was sampled. The frozen sample of the liver was minced and digested in 5% KOH at 40 °C for 12-18 hours. Egg counts of three 1 ml portion of the suspension were determined by microscopic examination at 40x. The mean of these counts was then used to be the EPG liver.

2.6. Liver Granuloma Measurement:

Granuloma diameters were measured in histological sections. After portal perfusion, paraffin sections were routinely stained with HE for microscopic examination. In each histological section, 10 granulomas with visible central eggs were randomly selected; their diameters were measured at 10x magnification using calibrated ocular micrometer. The mean granuloma sizes for each rabbit in a group were averaged for statistical comparison.

2.7. Elisa

Schistosome-specific ELISA was performed using the following materials:

100 µl/well of the antigen, 100 µl/well of 1:100 rabbit's sera obtained by cardiac puncture at the time of perfusion, 100 µl/well of 1:3000 anti-IgM-HRP or 1:500 of anti-IgG –HRP and the substrate (OPD). OD₄₉₂ values were read as ELISA reader.

2.8. Analysis of Data

The Student's *t*-test was used to calculate the significance of the differences and a value of $P < 0.05$ was taken as significant.

3. RESULTS

3.1. Worm recovery

Infection of rabbits with *S. japonicum* cercariae (100 cercariae/ rabbit) resulted in a worm recovery of 60.66 ± 5.85 worm/ rabbit. The mean worm burden in Group A animals was 27.50 ± 5.00 worm / rabbit, producing a 54.66% reduction in challenge parasites ($P < 0.001$). In Group B animals, the recovery of challenge worms was 43.00 ± 4.24 worm/rabbit resulted in a percentage reduction of 29.11% ($P < 0.05$).

Table 1: Adult worm recoveries (worm burdens) and reduction rates (% protection) after challenge infection

Group	Infection status	Worm burden	Protection	P- value
A	Congenital+ challenge	27.50 ± 5.00	54.66	< 0.001
B	Exposed+ challenge	43.00 ± 4.24	29.11	< 0.05
C	Challenge control	60.66 ± 5.85		

3.2. Liver egg counts

The mean number of eggs deposited in the liver in the challenge control rabbit was found to be 800.66 ± 19.00 EPG. It was 486.75 ± 42.69 in Group A, showing a

highly significant decrease of 41.45 % in the total liver eggs ($P < 0.001$). Group B showed a 25.37 % decrease in the total egg load compared with that in the challenge control rabbit ($P < 0.01$).

Table 2: Geometric mean number of tissue egg counts and the percentage reduction in challenge egg deposition in the liver

Group	Infection status	Mean EPG	Reduction	P-value
A	Congenital+ challenge	468.75 ± 42.69	41.45	< 0.001
B	Exposed+ challenge	597.50 ± 38.89	25.37	< 0.01
C	Challenge control	800.66 ± 19.00		

3.3. Granuloma size:

The mean size of granulomas in Group A animals was 49.30 ± 6.68 µm, not compared with 102.2 ± 17.14 µm in

the challenge controls ($P < 0.01$) whereas in Group B was 63.90 ± 4.10 µm resulted in a 37.48 % reduction in granuloma size ($P < 0.05$), Table 3.

Table 3: Granuloma sizes in different groups of rabbits 6 weeks after a single dose of 100 cercariae/ rabbit

Group	Infection status	Granuloma size (μm)	Reduction	P-value
A	Congenital+ challenge	49.30 $\pm$ 6.68	51.76	< 0.01
B	Exposed+ challenge	63.90 $\pm$ 4.10	37.48	< 0.05
C	Challenge control	102.2 $\pm$ 17.14		

3.4. Immunological findings

6 weeks after challenge infection, the mean IgM response to schistosome antigen in Group A was found

to be 0.8293 ± 0.021 ($P < 0.001$) whereas in Group B was 0.9285 ± 0.016 ($P < 0.05$). Comparing with Group C animals which exhibited the highest absorbance (1.3660 ± 0.13), Table 4.

Table 4: Mean OD_{492nm} values for IgM responses to (SEA +AWA)

Group	Infection status	Mean OD _{492nm}	Reduction (%)	P-value
A	Congenital+ challenge	0.8293 $\pm$ 0.21	39.47	< 0.01
B	Exposed+ challenge	0.9285 $\pm$ 0.016	32.03	< 0.05
C	Challenge control	1.3660 $\pm$ 0.13		

3.5. IgG responses

Group B-kittens showed a 18.75 % increases in the specific IgG antibody response ($P < 0.05$). Group A-

kittens displayed further increase in this response (56.22 % increment; $P < 0.001$), Table 5.

Table 5: Mean OD_{492nm} values for IgG responses to (SEA+AWA) preparation in the different groups of rabbits

Group	Infection status	Mean OD _{492nm}	Increment rate (%)	P-value
A	Congenital+ challenge	0.9748 $\pm$ 0.07	56.22	< 0.001
B	Exposed+ challenge	0.741 $\pm$ 0.006	18.75	< 0.05
C	Challenge control	0.624 $\pm$ 0.03		

4. DISCUSSION

Rabbits with an *S. japonicum* congenital infection 16 weeks previously showed a 54.66% resistant against the challenge infection. This is in keeping with the study of Willingham et al. (1997) who concluded that the challenge infection of pigs did not result in higher worm burden and higher tissue egg counts compared to the primary control group or the challenge control group. Attallah et al. (2006) demonstrated that adult worm count was significantly reduced in the offspring of *Schistosoma*-infected mothers compared to mice born to non-infected ones, but no explanation was offered. One possible reason for the presence or absence of severe pathology is prenatal exposure to schistosomiasis that modulates the future immune response later in life (Malhotra et al., 1997). This notion has been suggested by many authors and has been explored in experimental models (Camuset al., 1976; Montesano et al., 1999; King 2001). This could be explained by the concept of development of concomitant immunity (Hagan, 1996) in

which continuous antigenic stimulation (by the presence of some adult worms) stimulates protective immune response against further infections. Early stimulation of the immune system by in utero exposure to schistosomiasis may, therefore, provide quite strong protective immunity against initial postnatal infections. In sum, congenital exposure to *Schistosoma* infection seems to reduce the intensity of infection in subsequent postnatal exposure.

The congenitally-infected group also had a lower proportion of tissue eggs suggests that the previous infection status are able to destroy liver deposited eggs fairly rapidly. As considered by Domian et al. (1976) this could be due to the host's immunological response interfering with the uptake of essential nutrients by the parasite.

The present results show that the challenge infection reduces the magnitude of granulomatous responses of a postnatal challenge infection. The same observation had been made by Lewert and Mandlowitz (1969); Hang et al. (1974); Montesano et

al.(1999); Stavitsky (2004) and Attallah et al. (2006). The reduced size of granulomas observed by many authors and in our experiment in congenitally exposed kittens may be attributed to attenuated Th2 response. The latter may be due to, at least in part, an increase in counter-regulatory cytokines such as IL-12 and TGF- β or could be explained by an induction of immunological tolerance (Alves-Oliveira et al., 2006; Chu et al., 2007; Ahmad et al., 2010).

The present study shows that the congenital infection increases the IgM⁺ and IgG antibody titers compared to the exposed kittens. Modification of the immune response to schistosomal antigens, which may constitute the basis for these differences in this experiment, has been demonstrated in children born to *S. mansoni*-infected mothers. Attallah et al. (2006) found that Schistosoma-specific IgG levels were lower in prenatally exposed mice compared to control. This in contrast to Ahmad et al. (2010) who observed a highly significant increase in total IgG in offspring born to *S. mansoni*-infected mothers compared to offspring born to non-infected ones. Because it was not clear what kind of antibody and how antibody is needed for protection, immunological analysis of its protection by the congenital infection would be necessary for future study.

To our knowledge, this is the first report to investigate the *S. japonicum*/rabbit model showing the effect of congenital infection on these variables. The findings from the present study might have wide implications for the regulatory response to infection and the associated clinical and pathological consequences of *S. japonicum* infection and it may assist in further exploration of mechanisms and elucidation of causative role of *S. japonicum* infection in the mammalian host.

These results can be relevant to humans since many children are indeed born to infected mothers in endemic areas who are rarely manifest acute manifestations such as acute dermatitis or katayama fever (Caldas et al., 2008). This will add to the complexity of the situation, and might provide a stronger background for immunomodulation in subsequent infections.

In conclusion, congenital exposure to *S. japonicum* infection affects the immune response and the disease outcome in the future postnatal infections; the intensity of the infection as well as the immunopathological changes are moderated in congenitally exposed kittens. Finally, these results may give us insight for prevention and control strategies in schistosomiasis and perhaps in other chronic infectious diseases as well.

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