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Fecundity reduction of BALB/c mice after survival from lethal *Neodiplostomum seoulense* infection

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Abstract *Neodiplostomum seoulense* (Digenea: Neodiplostomidae), an intestinal trematode infecting humans and rodents, is known to be highly pathogenic and lethal to experimentally infected mice. Only a small proportion of mice can survive from its infection. This study aimed to assess the reproductive capacity of surviving BALB/c mice. The fertility of male and female mice, birth time (period from mating to birth of litters), number of litters, size and weight of testes or ovary-oviduct-uterus, apoptosis of testicular cells, and serum levels of sex hormones were determined. Our results revealed that surviving mice underwent severe fecundity reduction and finally became infertile. They could not be able to produce generations beyond F4. Fertility rate, birth time, and number of litters of *N. seoulense*-infected mice were all significantly ($p < 0.05$) lower than those of uninfected controls, *Metagonimus miyatai* (less pathogenic intestinal trematode)-infected, or castor oil (severe diarrheal agent)-administered controls. The size and weight of testes or ovary-oviduct-uterus were markedly ($p < 0.05$) decreased after *N. seoulense* infection. Moreover, the number of apoptotic cells in the testicular tissue was significantly ($p < 0.05$) increased (up to 10–50-folds) during weeks 1–3 post-infection. Serum testosterone levels in infertile mice were reduced to 1/10 level of fertile

mice. These results indicated that BALB/c mice surviving *N. seoulense* infection underwent destruction and apoptosis of gonad tissues with fecundity reduction. They were finally infertile, with no ability to produce their next generations.

Keywords *Neodiplostomum seoulense* · Intestinal fluke · Fecundity reduction · Infertility · Gonad degeneration

Introduction

Neodiplostomum seoulense (Digenea: Neodiplostomidae) (formerly *Fibricola seoulensis* Seo et al., 1964) is a highly pathogenic intestinal trematode of humans and rodents. It was originally described from a house rat in the Republic of Korea (=Korea) (Seo 1990). The first reported patient (a 25-year-old young man) complained of severe gastrointestinal troubles, fever, and eosinophilia. He consumed viscera of undercooked snakes several days before (Seo et al. 1982). Subsequently, more than 26 additional human cases were diagnosed in Korea (Hong et al. 1984, 1986).

Minute intestinal trematodes such as heterophyids (<2.0 mm in body length) generally elicit mild clinical symptoms unless the host is immunocompromised or heavily infected (Chai et al. 2005, 2009). Larger intestinal flukes such as echinostomes (0.5–17.7 mm long) can induce more severe clinical manifestations (Chai 2009). However, although *N. seoulense* is minute in size (0.8–1.2 mm in length), it can cause severe gastrointestinal symptoms, including cramping abdominal pain and lethargy (Seo 1990). Mice experimentally infected with *N. seoulense* have revealed severe destruction of intestinal villi, crypt hyperplasia, mastocytosis, and goblet cell hyperplasia in the mucosa of their small intestines (Lee et al. 1985; Chai et al. 1998). *N. seoulense* infection has never been found in other body sites, including male and female gonads.

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A striking observation in *N. seoulense* infection was that ICR mice given 1000 metacercariae died by day 16 post-infection (PI), indicating that a heavy *N. seoulense* infection is lethal to mice (Huh et al. 1988). It was further reported that various strains of mice (C3H/HeJ, BALB/cA, C57BL/6, mast cell-deficient W/W^v, and its littermates +/+) infected with even lower infection dose (200 metacercariae) also died by day 23–29 PI (Kook et al. 1998). The precise reasons for the death of mice remain to be elucidated. However, intestinal paralysis (Kook et al. 1998) presumably due to immune system disturbance (e.g., dominant Th1 response) has been suggested as one of the reasons.

The susceptibility of mice to this lethality is dependent upon the infection burden and the genetic background of mice (Kook et al. 1998; Chai et al. 2000). Whereas all C3H/HeJ mice died by day 29 PI (200 metacercariae), up to 26.7 % of C57BL/6 mice and 86.7 % of BALB/c mice survived with severe weakness and recovery after day 28 PI (Kook et al. 1998). The fatality of mice due to *N. seoulense* infection has been suggested to be influenced by different levels of immune responses determined by the genetic background of mice, e.g., H-2 gene products (Chai et al. 2000). Studies using BALB/c mice would be instructive for understanding these relationships.

The F1 generation of fatality-susceptible (C3H/HeN) and fatality-resistant (BALB/c) mice showed an intermediate level of survival (Kook et al. 1998), suggesting that it might be possible to create a sub-strain of BALB/c mice with the capacity to resist *N. seoulense*-related fatality by mating surviving male and female BALB/c mice through several generations. Thus, we attempted to produce F1, F2, F3, and F4 generations of BALB/c mice that might be resistant to fatality due to *N. seoulense* infection. However, the attempt was failed because the majority of surviving BALB/c mice after *N. seoulense* infection underwent fecundity reduction with final infertility. We observed the changes in fertility rate, birth time (period from mating to birth of litters), litter size, size and weight of testes or ovary-oviduct-uterus, the number of apoptotic cells in the testes, and serum testosterone and estradiol levels in BALB/c mice surviving *N. seoulense* infection. Some of these data were compared to those of *Metagonimus miyatai* (less pathogenic intestinal trematode)-infected or those with castor oil (severe diarrheal agent)-administered control mice.

Materials and methods

Animals

Male and female BALB/c mice raised under specific pathogen-free (SPF) conditions were purchased from the Korean Animal Center (Jinju, Korea). They were 6–7 weeks old at the beginning of the experiment. They were maintained in a SPF animal facility at Seoul National University College

of Medicine (Seoul, Korea) until used. Animal experiments were carried out in accordance with the guidelines issued by the Institutional Animal Care and User Committee, Seoul National University College of Medicine, Seoul, Korea.

Experimental groups

The following three experimental groups were prepared: (1) *N. seoulense*-infection group (0, 100, and 200 metacercariae infection subgroups; 10–15 mice/subgroup), (2) *M. miyatai*-infection group (0, 200, and 500 metacercariae infection subgroups; 4–10 mice/subgroup), and (3) castor oil administration group (no castor oil and castor oil subgroups; 8 mice/subgroup). The *M. miyatai*-infection group was included to determine whether the infertility of BALB/c mice also occurred after *M. miyatai* infection. The castor oil control group was used to investigate whether induction of severe fatal diarrhea could cause infertility of BALB/c mice.

Collection of parasites and infection to mice

Snakes (*Rhabdophis tigrina*) naturally infected with metacercariae of *N. seoulense* were purchased from Hoengseong, Gangwon-do Province, Korea, an enzootic area of this fluke. They were maintained at 4 °C until required. Pale chubs (*Zacco platypus*), the fish host for *M. miyatai*, were purchased from a market in Yeongweol-gun, Gangwon-do, Korea. The metacercariae of *N. seoulense* were collected from the mesentery and viscera of snakes. Those of *M. miyatai* were isolated from the scale of chubs. Artificial gastric juice containing 0.5 % pepsin (1:10,000, Sigma Chemical Co., St. Louis, MO, USA) and 0.8 % HCl was used to isolate metacercariae as described previously (Chai et al. 2000). BALB/c mice were infected orally with *N. seoulense* (100 or 200) or *M. miyatai* (200 or 500) metacercariae in 0.2 ml of saline using a gavage needle.

Administration of castor oil

In order to induce fatal diarrhea, BALB/c mice were orally administered with 2 ml of castor oil (Sigma Chemical Co., St. Louis, MO, USA) per mouse. Their clinical courses were observed. About 20 % of castor oil-administered mice were dead by day 14 post-administration. Surviving male and female BALB/c mice were mated to produce F1 mice.

Assessment of survival and fertility of mice

The survival of BALB/c mice infected with *N. seoulense* or *M. miyatai* was observed for 5 weeks after the infection. Surviving mice were allowed 3 to 5 weeks of recovery period before assessing fertility. Five weeks plus 3–5 weeks were considered to be sufficient for full recovery from

N. seoulense or *M. miyatai* infection. Almost no flukes were found to retain in their small intestines around this time. If the surviving male and female mice produced litters (by placing a male and a female in one cage), they were judged as fertile. If they did not produce litters during the 2 months period, the female was taken out and put in a new cage with a normal uninfected male mouse. If this female did not become pregnant during the next 2 months, this female was judged as infertile. Similar assessment methods were applied for males.

Assessment of delayed birth time and decreased litter size

The period from mating of a male and a female BALB/c mouse to delivery of their litters (birth time) was measured for each mouse cage. Litter size was checked when the delivery was completed.

Measurement of size and weight of testes or ovary-oviduct-uterus of mice

The testes and ovary-oviduct-uterus of male and female BALB/c mice infected with *N. seoulense* (weeks 0, 1, 2, 3, 4, 5, and 6 PI) were excised. Their length, width, and weight were measured. Mice of week 0 were denoted as uninfected controls.

Measurement of apoptotic cells in the testes

The testes of BALB/c mice infected with *N. seoulense* (0, 100, and 200 metacercariae subgroups) were removed at weeks 1, 2, and 3 PI. They were processed for detection of gonad cell apoptosis. The degree of apoptosis was measured by transferase-mediated dUPT nick end-labeling (TUNEL) method using In Situ Apoptosis Detection Kit (Intergen, Manhattanville, NY, USA). Briefly, removed testes were fixed in 10 % neutral-buffered formalin, sectioned to about 5 μ m in thickness, and stained using the kit. Fragmented genomic DNA was labeled with digoxigenin-TdT (terminal deoxynucleotidyl transferase) and colorized with DAB (3,3'-diaminobenzidine). The total number of apoptotic cells in a section of a whole testis was counted and compared according to weeks (1–3 weeks) after infection.

Measurement of serum sex hormone levels

Serum levels of testosterone and 17 β -estradiol were measured in male and female BALB/c mice, respectively, after infection with 200 metacercariae of *N. seoulense*. Mice surviving the infection were later judged as either fertile or infertile depending on the results of mating experiment (placing a male and a female in a cage). Serum sex hormones were measured respectively. Briefly, whole blood was collected by cardiac puncture under ether anesthesia. It has been reported

previously that such sampling procedures will not alter serum values of steroid hormones such as progesterone, androgen, or estrogen (Matt et al. 1986). Blood was allowed to clot at room temperature for about 1 h, kept at 4 °C for several hours, and centrifuged. Sera were separated and stored at –20 °C until assay. The concentrations of testosterone and 17 β -estradiol were determined using Testosterone EIA kit and Estradiol EIA kit (Cayman, Ann Arbor, MI, USA), respectively, according to the manufacturer's protocols.

Statistical analysis

Data were presented as mean $\pm$ standard deviation (SD). Statistically significant differences in fertility rate, birth time, litter size, size and weight of gonads, and sex hormone levels were determined using the Student's *t* test. The *p* values of less than 0.05 were considered as statistically significant.

Results

Failure to reproduce mouse generations

The attempt to produce fatality-resistant BALB/c mice beyond F4 generation was failed because the majority of surviving BALB/c mice after *N. seoulense* infection underwent fecundity reduction with final infertility (Table 1). The fertility rate of male and female mice never exceeded 50 % and was progressively decreased to finally become 0 % at F4 generation.

Fertility rate of mice

The fertility rate of *N. seoulense* uninfected control mice was 100 % for both males and females (Fig. 1). However, the fertility rate was decreased significantly ($p < 0.05$) when mice were infected with 100 (66.0 % for males and 57.0 % for females) or 200 metacercariae of *N. seoulense* (37.5 % for males and 42.8 % for females) (Fig. 1). The gender difference was not statistically significant ($p > 0.05$). In *M. miyatai*-infection or castor oil administration groups, the fertility rate was not changed. It was maintained at 100 % (Fig. 1).

Birth time

The birth time of female mice was 23.2 ± 1.6 days for *N. seoulense*-uninfected controls (Fig. 2). However, this value was increased significantly ($p < 0.05$) to 41.3 ± 16.7 days and 58.7 ± 7.8 days when mice were infected with 100 and 200 metacercariae of *N. seoulense*, respectively (Fig. 2). In *M. miyatai*-infected mice, the birth time was not significantly ($p > 0.05$) altered (26.4 ± 5.0 days in

Table 1 Survival and fertility of several generations of BALB/c mice infected with 200 metacercariae of *N. seoulense*

		Generation of mice				
		Parent	F1	F2	F3	F4
No. of infected mice	Male	10	4	4	13	2
	Female	10	14	15	17	2
	Total	20	18	19	30	4
No. (%) of mice survived	Male	5 (50.0)	1 (25.0)	3 (75.0)	11 (84.6)	2 (100.0)
	Female	5 (50.0)	10 (71.4)	13 (86.7)	14 (82.4)	1 (50.0)
	Total	10 (50.0)	11 (61.1)	16 (84.2)	25 (83.3)	3 (75.0)
No. (%) of fertile mice	Male	2 (40.0)	1 (100.0)	1 (33.3)	1 (9.1)	0 (0.0)
	Female	3 (60.0)	3 (30.0)	2 (15.4)	1 (7.1)	0 (0.0)
	Total	5 (50.0)	4 (36.4)	3 (18.8)	2 (8.0)	0 (0.0)
Litter size	Male	4	4	13	2	0
	Female	14	15	17	2	0
	No./female	6.0	6.3	15.0	4.0	0.0

200 metacercariae subgroup and 25.0 ± 1.0 days in 500 metacercariae subgroup). In castor oil-administered mice, the birth time was slightly increased to 35.5 ± 7.8 days compared to mice without castor oil administration. However, such increase was not statistically significant ($p > 0.05$).

Litter size

The litter size was 8.8 ± 0.8 for *N. seoulense* uninfected controls (Fig. 3). The litter size decreased significantly ($p < 0.05$) to 6.0 ± 0.8 and 3.7 ± 0.6 when mice were

infected with 100 and 200 metacercariae, respectively (Fig. 3). For *M. miyatai*-infected mice, the litter size in 200 or 500 metacercariae subgroups was not significantly ($p > 0.05$) altered compared to that of uninfected controls. For the group administered with castor oil, the litter size was decreased slightly. However, such decrease was not statistically significant ($p > 0.05$).

Decrease in size and weight of testes of male mice

The testes and ovary-oviduct-uterus of male and female BALB/c mice infected with *N. seoulense* (100 or 200

Fig. 1 Decreased fertility rate of BALB/c mice (male and female) recovered from *N. seoulense* infection compared to that of control mice infected with *Metagonimus miyatai* (a less pathogenic intestinal trematode) or administered with castor oil (a diarrheal agent). The decrease in fertility in *N. seoulense*-infected mice (200 metacercariae subgroup) was statistically significant ($p < 0.05$ for both male and female mice) compared to that of the controls

Fig. 2 Prolonged birth time (period from mating to birth of litters) in female BALB/c mice recovered from *N. seoulense* infection compared to that of controls infected with *Metagonimus miyatai* or administered with castor oil. The prolongation in birth time in *N. seoulense*-infected mice was statistically significant ($p < 0.05$) in both 100 and 200 metacercariae infection subgroups compared to that of the controls. Data represent the mean $\pm$ SD of 2–7 mice per subgroup

metacercariae) underwent remarkable decreases in their size (length and width) and weight (Figs. 4 and 5). The length and width of the right testis in uninfected control mice ($n=2$) was 8.1 ± 0.1 mm and 5.3 ± 0.2 mm, respectively. The left testis was 8.2 ± 0.2 mm in length and 5.6 ± 0.1 mm in width. Remarkable ($p < 0.05$) decreases were noted in the length and width of the right testis of *N. seoulense*-infected mice from week 4 PI (Fig. 4). The

left testis showed the same decreasing pattern (data not shown). Similarly, the weight of testes was also notably decreased after *N. seoulense* infection. The weight of the right and left testes of uninfected control mice were 90.0 ± 0.0 mg and 95.0 ± 7.1 mg, respectively. The weight of the right and left testes of *N. seoulense*-infected mice was decreased remarkably ($p < 0.05$) from week 4 PI. It weighed less than half of the control at week 6 PI (Fig. 4).

Fig. 3 Decreased litter size in female BALB/c mice recovered from *N. seoulense* infection compared to that of controls infected with *Metagonimus miyatai* or administered with castor oil. The decrease in litter size was statistically significant ($p < 0.05$) in *N. seoulense*-infected mice (100 or 200 metacercariae) compared to the controls. The litter size was also decreased in castor oil-administered mice. However, such decrease was not statistically significant ($p > 0.05$). Data represent the mean $\pm$ SD of 2–7 mice per subgroup

Testes

Fig. 4 Decrease in size (*length* and *width*) and weight of testis in BALB/c mice recovered from *N. seoulense* infection (100 metacercariae). The decreases in testis length, width (*right testis*), and

weight (both testes) were statistically significant ($p < 0.05$) from week 4 to 6 post-infection (PI). Data represent the mean $\pm$ SD of 2–3 mice per week

Ovary-oviduct-uterus

Fig. 5 Decrease in size (*length* and *width*) and weight of the ovary-oviduct-uterus in BALB/c mice recovered from *N. seoulense* infection (100 metacercariae). The decreases in size and weight were statistically

significant ($p < 0.05$) from week 2 to 6 PI. Data represent the mean $\pm$ SD of 2–3 mice per week

Fig. 6 Testicular cell apoptosis in *N. seoulense*-infected BALB/c mice based on TUNEL method, $\times 400$. **a** Brown-colored apoptotic germ cells were seen in the testicular cord, week 1 PI. **b** An increased number of brown-colored apoptotic germ cells were seen at week 2 PI. **c** Numerous apoptotic germ cells were gathered near the peripheral part of the seminiferous tubule, week 3 PI

Decrease in size and weight of ovary-oviduct-uterus of female mice

With regard to ovary-oviduct-uterus, their length and width in uninfected control mice ($n=2$) were 2.0 ± 0.3 mm and 3.2 ± 0.0 mm, respectively. They were decreased significantly ($p < 0.05$) in *N. seoulense*-infected mice (100 metacercariae subgroup) from week 1 PI until week 6 PI (Fig. 5). The weight of the ovary-oviduct-uterus was significantly ($p < 0.05$) decreased after *N. seoulense* infection. Its weight in uninfected control mice was 145 ± 7.1 mg. The weight of the ovary-oviduct-uterus in *N. seoulense*-infected mice was decreased remarkably ($p < 0.05$) from week 3 PI. It weighed less than one third of its control weight at weeks 4–6 PI (Fig. 5).

Apoptosis in testicular tissue

Histologically, the testes of *N. seoulense*-infected BALB/c mice (100 or 200 metacercariae) underwent severe degeneration, showing apoptosis of gonad cells during weeks 1–3 PI (Figs. 6 and 7). The number of apoptotic cells in a section of whole testicular tissue in uninfected control mice was minimal (1.4 ± 1.1). It was increased 10- and 30-folds in 100 and 200 metacercariae subgroups, respectively, at week 1 PI (Fig. 7). The increase of apoptotic cells was more pronounced at weeks 2–3 PI (30- and 50-folds in 100 and 200 metacercariae subgroups, respectively, Fig. 7).

Serum sex hormone concentrations

Serum testosterone levels in infertile male mice were markedly lower (10-fold, $p < 0.05$) than those in fertile male mice (Tables 1 and 2). Estrogen (17- β estradiol) concentrations in infertile female mice were lower than those in fertile females. However, the difference was not statistically significant ($p > 0.05$, Table 2).

Discussion

After infection with *N. seoulense*, both male and female BALB/c mice exhibited decreased fertility as well as prolonged birth time, decreased litter size, reduced size and weight of testes and ovary-oviduct-uterus, increased apoptotic cells in gonads, and decreased serum sex hormone levels. The fecundity reduction was infection intensity-dependent and progressive. It appeared to be irreversible. It was induced only by *N. seoulense* infection, not by *M. miyatai* infection or castor oil administration. Mechanical castration of male or female gonads by direct invasion of parasites is not applicable for *N. seoulense* infection (Seo 1990). Moreover, the possibility of creating a sub-strain of BALB/c mice resistant to the fatality caused by *N. seoulense* infection turned out to be a failure due to fecundity reduction of surviving mice after the infection.

Parasite-induced host reproductive failure has been reported in a wide range of parasite and host species

Fig. 7 Increase in the number of apoptotic germ cells in the testis of BALB/c mice recovered from *N. seoulense* infection (100 or 200 metacercariae). The number was increased progressively from week 1 to 3 PI. Data represent the mean $\pm$ SD of five mice/week/each infection dose

Table 2 Serum sex hormone concentrations in *N. seoulense*-infected male or female mice

Hormone	Status of reproduction	Concentration (pg/ml)	
		Male	Female
Testosterone	Fertile ($n=5$)	6344.0 $\pm$ 2789.0*	548.0 $\pm$ 582.0
	Infertile ($n=5$)	628.0 $\pm$ 468.0*	129.0 $\pm$ 109.0
17- β estradiol	Fertile ($n=5$)	21.4 $\pm$ 6.8	56.0 $\pm$ 23.8
	Infertile ($n=5$)	23.0 $\pm$ 10.5	23.8 $\pm$ 6.9

* $p < 0.05$, significantly different between fertile and infertile male mice

(Hurd 2001; Bonds 2006; Lafferty and Kuris 2009). This reproductive failure is an evolutionary strategy of the parasite or the host (Bonds 2006). This can be classified into two broad categories (Lafferty and Kuris 2009). One is the so-called parasitic castration, a parasitic strategy that requires the eventual intensity-independent elimination of host reproduction as the primary means for acquiring energy (Nagano 1966; Lafferty and Kuris 2009; Lafevre et al. 2009; Faro et al. 2013). The other is host fecundity reduction (some authors use this term broadly to include parasitic castration). It is a host strategy to limit damages caused by parasites which results in more nutrients and energy available for the host for its survival (Hurd 2001; Lafferty and Kuris 2009). In some instances, it is difficult to distinguish host fecundity reduction from parasitic castration (Lafferty and Kuris 2009).

In parasitic castration, even a single individual castrator can prevent or block host reproduction (Lafferty and Kuris 2009). Such parasite can take all the reproductive energy of the host (Bonds 2006). Common parasitic castrators include larval trematodes in snails, isopod and barnacle parasites of crustaceans, a few insects, nematode, and Platyhelminthes species (Lafferty and Kuris 2009). In host fecundity reduction, the host can temporarily divert its reproductive energy into defense to tackle the infection more effectively (Hurd 2001). It can also be a side effect of pathology (Lafferty and Kuris 2009). Host fecundity reduction is infection intensity-dependent. Therefore, individual parasites are not castrators (Lafferty and Kuris 2009). This phenomenon has been reported in a variety of vertebrate hosts, including mice (Stahl et al. 1994), deer (Formenti et al. 2015), and marsupials (Fancourt et al. 2014) infected with *Toxoplasma gondii*, mice infected with *Leishmania major* (Krishnan et al., 1996), squirrels infected with fleas (Neuhaus 2003), and human population infected with hookworms (Blackwell et al. 2015).

The reproductive failure of male and female BALB/c mice surviving *N. seoulense* infection shown in this study seemed to be a type of host fecundity reduction resulting from host strategy to overcome the fatal *N. seoulense* infection. It was infection intensity-dependent and involved a

vertebrate host. Hence, it did not fit the parasitic castration strategy in a strict sense. This type of host fecundity reduction has been reported in nematode, cestode, protozoan, or ectoparasite infections but never in trematode infections (Rikihisa et al. 1985; Stahl et al. 1994; Krishnan et al. 1996; Neuhaus 2003; Blackwell et al. 2015). In nematodes, human hookworm infections were positively associated with delayed first pregnancy and extended interbirth intervals among women living in the Amazonian lowlands of Bolivia (Blackwell et al. 2015). In cestodes, serum testosterone level was reported to be significantly lowered in *Taenia taeniaeformis* metacestode-infected rats, mediated by excretory-secretory product of metacestode (Rikihisa et al. 1985). With regard to protozoans, female mice chronically (1–2 months) infected with a cyst-forming Cornell strain of *T. gondii* were reported to have reproductive failure with cessation of estrus cycling and constant diestrus vaginal cytology (Stahl et al. 1994). This reproductive failure has been suggested to be due to acquired hypogonadotrophic hypogonadism secondary to hypothalamic dysfunction (Stahl et al. 1994). Another example of protozoan infection is *L. major* in C57BL/6 mice that exhibited implantation failure and fetal resorptions (Krishnan et al. 1996).

The molecular or immunological mechanism of fecundity reduction in vertebrate hosts infected with parasites remains to be clarified. However, immunopathogenesis including immune imbalance has been suggested to be one of the reasons. Whereas a beneficial anti-parasite Th1 response in C57BL/6 mice infected with *L. major* can adversely affect pregnancy outcome, in terms of placental maintenance as well as preimplantation events, Th2 response did not reduce implantations in BALB/c mice and succumbed to the infection (Krishnan et al. 1996). In *N. seoulense* infection, both Th1 and Th2 responses occur during the course of infection in BALB/c mice (Shin et al. 2007). It is suggested that these mixed Th1 and Th2 immune responses synergistically induce host damage and worm expulsion (Shin et al. 2007). However, Th1 response may play more significant roles than Th2 response in immunopathogenesis leading to host damage (Shin et al. 2007). Host fecundity reduction after *N. seoulense* infection may also become a consequence of overexpression of Th1 response. However, this needs verification.

In conclusion, *N. seoulense*, an intestinal trematode infecting humans and rodents in Korea, was found to suppress host fecundity. Our attempt to produce a sub-strain of BALB/c mice resistant to the fatality by *N. seoulense* infection failed. This type of host fecundity reduction differs from parasitic castration that is frequently seen in invertebrate hosts. The host fecundity reduction in *N. seoulense*-infected mice appeared to be due to host strategy to overcome the severe damage caused by the fatal parasitic infection. Possible mechanisms related to this strategy such as immune imbalances need further elucidation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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