Intestinal immunity suppresses carrying capacity of rats for the model tapeworm, *Hymenolepis diminuta*

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Abstract

Hymenolepis diminuta is a parasitic tapeworm of the rat small intestine and is recognized as a useful model for the analysis of cestode-host interactions. In this study, we analyzed factors affecting the biomass of the tapeworm through use of rat strains carrying genetic mutations, namely X-linked severe combined immunodeficiency (*xscid*; T, B and NK cells deficiency), nude (*rnu*; T cell deficiency), and mast cell deficient rats. The worm biomass of F344-*xscid* rats after infection with 5 cysticercoids was much larger than control F344 rats from 3 to 8 weeks. The biomass of F344-*rnu* rats was also larger than the controls, but was intermediate between F344-*xscid* and control rats. These observations demonstrated that host immunity can control the maximal tapeworm biomass, i.e., carrying capacity, of the rat small intestine. Both T cell and other immune cells (B and NK cells) have roles in determining the carrying capacity of tapeworms. Total worm biomass and worm numbers in mast cell deficient rats (WsRC-*Ws/Ws*) were not significantly different from control WsRC-+/+ rats after 3 and 6 weeks of primary infection. Mast cell deficient rats displayed reinfection resistance for worm biomass but not worm expulsion. These findings suggest that the mast cell has a role for controlling the biomass of this tapeworm in reinfection alone, but does not affect the rate of worm expulsion. Overall, our findings indicate that the mast cell is not a major effector cell for the control of the carrying capacity of tapeworms. The identity of the major effector cell remains unknown.

Keywords: carrying capacity, *Hymenolepis diminuta*, immune-deficient rat, mast cell deficient rat, worm biomass

1. Introduction

The tapeworm *Hymenolepis diminuta* is a chronic and asymptomatic parasite living in the small intestine of rats. It has a relatively simple life cycle and is easily maintained in the laboratory using rats (definitive host) and flour beetles (intermediate host). *H. diminuta* is recognized as a useful model for the analysis of cestode-host interactions, especially for elucidating the complexity of the mammalian immune system response to extra-cellular large intestinal helminths [1-3]. The rat small intestine has a capacity limit after *H. diminuta* infection with regard to worm biomass, the so-called carrying capacity [3]. When the number of worms is low, each individual is larger than those obtained from rats harboring larger numbers of worms [3]. Carrying capacity is limited by inter-worm competition for space and nutrients [3] in the definitive host. Although rats show an immune response against *H. diminuta* [1, 3-5], it is not certain whether this affects the carrying capacity of the small intestine.

H. diminuta chronically infects the rat small intestine, but is spontaneously expelled from that of mice (non-definitive host) [3]. A few immune-deficient models produced by genetic manipulation have been utilized to investigate factors that cause expulsion of *H. diminuta* in mice [2,3]. Although only *STAT6* knockout mice can harbor adult tapeworms [6], the worm biomass in this case may not suitable for investigation of effects on carrying capacity for this tapeworm. The carrying capacity needs to be evaluated in the definitive host (rat). Recently X-linked severe combined immune-deficiency (*xscid*) rats with markedly diminished numbers of T, B and NK cells were established on an F344 genetic background by genome editing technology [7]. There is a clear strain dependent variation in tapeworm persistence and biomass after *H. diminuta* infection, with F344 being a susceptible strain [8]. In this study, we evaluated the effects of host immune response on the carrying capacity for

this tapeworm in the guts of rats which carrying genetic mutations on an F344 genetic background; specifically, we investigated X-linked severe combined immune deficiency and nude rats (which have a marked reduction in T cells) [9]. Although mucosal mast cells have no direct role in the expulsion of *H. diminuta* [10, 11], the role of this cell type in tapeworm persistence and biomass is not well understood. Therefore, we also studied the function of mast cells on carrying capacity by using mast cell deficient Kit^{Ws}/Kit^{Ws} rats that have severely impaired *c-kit* kinase activity as a result of a 12 bp deletion in the tyrosine kinase domain of *c-Kit* [12-14]. Reinfection resistance with respect to worm expulsion and inhibition of worm growth has been reported previously after secondary infection of rats with *H. diminuta* [1, 15]. Therefore, we also examined the role of mucosal mast cells in reinfection resistance in the Kit^{Ws}/Kit^{Ws} rats.

2. Materials and methods

2.1 Rats

F344-$Il2rg^{em2Kyo}$ (F344-*xscid*) rats (NBRP Rat No: 0586), which show X-linked severe combined immune deficiency and have a genetically disrupted interleukin-2 receptor gamma gene (*Il2rg*) [7], were supplied by the National BioResource Project-Rat, Kyoto University (Kyoto, Japan). F344 rats were purchased from Charles River Laboratories Japan (Yokohama, Japan). F344.Cg-$Foxn1^{rnu}$ (F344-*rnu*) rats [9] were purchased from CLEA Japan (Tokyo Japan), and WsRC-Kit^{Ws}/Kit^{Ws} (WsRC-*Ws/Ws*), WsRC-+/+ and Wistar rats were purchased from Japan SLC (Hamamatsu, Japan). All rats were fed a commercial CE-2 diet (CLEA Japan, Tokyo) and had *ad libitum* access to water. The rats were bred in a pathogen-free facility at the Institute for Laboratory Animal Research, Graduate School of Medicine, Nagoya University, and maintained under a controlled temperature of 23 ± 1°C, humidity of 55 ± 10%, and a light cycle of 12-hour light (from 09:00 to 21:00)/12-hour dark (from 21:00

to 09:00). Animal care and all experimental procedures were approved by the Animal Experiment Committee, Graduate School of Medicine, Nagoya University, and were conducted according to the Regulations on Animal Experiments of Nagoya University.

2.2 Infection and maintenance of the tapeworm

H. diminuta is maintained by cyclical passage through Wistar rats and the flour beetle (*Tribolium confusum*). Flour beetles are fed gravid proglottids that develop into infective cysticercoids within 2 weeks; the infective cysticercoids remain dormant in the hemocoel of the beetle. Cysticercoids were recovered under the dissecting microscope from infected flour beetles at least 3 weeks after exposure to gravid proglottids. The cysticercoids were pooled, and administered immediately to the experimental rats. Seven-week-old female rats were orally administered a known number of cysticercoids through a stomach tube under light anesthesia using isoflurane inhalation. Infected rats were sacrificed, and the small intestine was removed from each rat. Tapeworms were carefully flushed out with saline, and the total number of worms and weights of each worm recovered from each rat were recorded.

2.3 Reinfection by tapeworm

Four weeks after primary *H. diminuta* infection with 10 cysticercoids, rats were treated with praziquantel (Sigma Chemical Co., St. Louis, USA), a very effective anti-cestodal drug [16, 17]. Isoflurane-anesthetized infected rats were orally administered praziquantel (50 mg/kg BW) on two consecutive days. Tapeworm infection in rats and the complete removal of worms were confirmed by fecal examinations before and 3 days after praziquantel treatment, respectively. One week after anti-cestodal treatment, the rats were reinfected with 5 cysticercoids.

2.4 Statistical analysis

The results are expressed as means and standard deviations. A one-way analysis of variance (ANOVA) and subsequent Bonferroni’s test was used to determine the significance

of differences in multi-group comparisons. Student's t-test was used to compare two-group differences. Differences with $P < 0.05$ were regarded as significant.

3. Results

The time course of changes in worm biomass after infection of rats with 5 cysticercoids is shown in Figure 1. In immune-deficient rats (F344-*rnu* and F344-*xscid*), the worm biomass was significantly larger than in control F344 rats from 3 to 8 weeks after infection. In a preliminary analysis at 3 weeks after infection, the number of eggs in the rat feces tended to increase in proportion to the worm biomass. Worm fragments with gravid proglottids from immune-deficient rats were longer and wider than those from normal rats. Total worm biomass in F344-*xscid* rats was more than three-fold greater than in controls at 8 weeks after infection; total worm biomass in F344-*rnu* rats was significantly larger than controls but less than in F344-*xscid* rats. Worm biomass in both F344-*nu* and F344-*xscid* rats increased to 6 weeks and remained at this level to 8 weeks after infection; by contrast, the worm biomass in F344 rats gradually decreased after 3 weeks. On the WsRC genetic background, there were no significant differences in worm biomass between mast cell deficient (WsRC-*Ws/Ws*) and control (WsRC-+/+) rats from 3 to 6 weeks after infection. Total worm biomass of both WsRC-+/+ and WsRC-*Ws/Ws* rats decreased after 3 weeks, in a similar manner as in normal F344 rats. The numbers of recovered worms from rats with F344 or WsRC genetic backgrounds did not differ significantly in the primary infection; most rats had 5 worms. All infected mutant rats (F344-*xscid*, F344-*nu*, WsRC-*Ws/Ws*) were asymptomatic without diarrhea or weight loss, similarly to normal rats.

Total worm biomass and mean worm weights at 3 weeks after infection with different numbers of cysticercoids are shown in Figure 2 for F344-*xscid* and control F344 rats. There were no significant differences in either total worm biomass or mean worm weight between

F344 and F344-*xscid* rats infected with 1 or 2 cysticercoids. Total worm biomass and mean worm weight in F344 rats were significantly lower than those of F344-*xscid* rats infected with 5 cysticercoids. In F344-*xscid* rats, total worm biomass tended to increase proportionally with the number of cysticercoids used for infection; by contrast, total worm biomass in F344 rats did not increase even when more than 2 cysticercoids were used for infection. Mean worm weights in both F344 and F344-*xscid* rats infected with 5 cysticercoids were significantly lower than those with 1 or 2 cysticercoid infection. In particular, the mean worm weight in F344 rats after 5 cysticercoid infection was much lower than that after 1 or 2 cysticercoid infection.

The changes in worm numbers in rats after primary and secondary infections at 3 weeks after infection with 5 cysticercoids are shown in Figure 3. The number of worms recovered after secondary infection of F344 rats with normal immunity was significantly lower than after primary infection due to reinfection resistance. By contrast, there were no changes in the numbers of recovered worms between primary and secondary infections in F344-*xscid* rats. In rats on a WsRC genetic background, worm numbers in control rats (WsRC-+/+) after secondary infection were significantly lower than after the primary infection. In mast cell deficient rats (WsRC-*Ws/Ws*), worm numbers did not differ significantly between primary and secondary infections. Worm numbers after secondary infection were not significantly different between control and mast cell deficient rats.

Changes in worm biomass between primary and secondary infections at 3 weeks after infection of rats with 5 cysticercoids are shown in Figure 4. Worm biomass in F344 rats after secondary infection was markedly lower than that after the primary infection; however, there were no changes in worm biomass between primary and secondary infections in F344-*xscid* rats. WsRC-+/+ rats showed similar worm biomasses in primary and secondary infections, although worm biomass after secondary infection tended to decrease. No changes in worm

biomass were observed between primary and secondary infections in mast cell deficient WsRC-*Ws/Ws* rats. Worm biomass after secondary infection in mast cell deficient rats was significantly higher than in control rats.

4. Discussion

In preliminary experiments and throughout this study, we did not find any tapeworms with a wet weight exceeding 1.0 gram. This suggests that the growth limit for this tapeworm is less than 1.0 gram in the intestines of F344 genetic background rats. The lack of a significant difference in tapeworm weight between F344-*xscid* and F344 rats after infection with 1 or 2 cysticercoids (Fig. 2) indicates that the rat intestine permitted tapeworms to grow close to their size limit when the total biomass was within the carrying capacity. Once total worm biomass reached the carrying capacity, for example in 5 cysticercoid infection, worms could not grow to their size limit due to a crowding effect. However, worms could grow to comparatively close to the size limit in F344-*xscid* rats after 5 cysticercoid infection because of the much heavier carrying capacity of these rats compared to F344 rats. As a result, a significant difference in mean worm weight was observed between F344-*xscid* and F344 rats after 5 cysticercoid infection (Fig. 2). Significant decreases in mean worm weights after 5 cysticercoid infection compared to 1 or 2 cysticercoid infection (Fig. 2) indicated that the total biomass reached carrying capacity in both F344 and F344-*xscid* rats after the 5 cysticercoid infection. This suggests that total worm biomass after 5 cysticercoid infection reflects the carrying capacity of the rat intestine for *H. diminuta.*

Our analysis clearly demonstrated that the carrying capacity for *H. diminuta* was suppressed by host immunity (Fig. 1). The increased carrying capacity of F344-*rnu* rats compared to F344 with normal immunity indicates that T cells act to suppress the carrying capacity for *H. diminuta*. Similarly, the clear increase in carrying capacity in F344-*xscid* rats

compared to F344-*rnu* shows that B and NK cells are also involved in the suppression of carrying capacity. Previously, it was reported that worm biomass is greatest at about 3 weeks after infection; biomass subsequently decreases in the normal rat intestine [11, 18, 19]. Neither F344-*nu* nor F344-*xscid* rats exhibited a gradual decrease of worm biomass until 8 weeks after infection (Fig. 1), whereas, control F344 rats exhibited a gradual decrease similar to that reported previously. We suggest that the gradual decrease in worm biomass after the peak is controlled by acquired immunity through T cells. Although the tapeworm and its eggs are asymptomatic in rats, it is possible that excessive growth of a tapeworm in the intestine inhibits the absorption of nutrients. Suppression of tapeworm biomass by host immunity might be a significant factor in the rat intestine.

The total worm biomass of mast cell deficient WsRC-*Ws/Ws* rats was not significantly different from that of control WsRC-+/+ rats from 3 to 6 weeks after infection (Fig. 1). Furthermore, the worm biomass of mast cell deficient rats decreased after 3 weeks (Fig. 1). These findings indicated that mast cells were not a major effector cell type for controlling the carrying capacity and the time-dependent decrease of *H. diminuta* biomass in the rat intestine. However, a significantly higher worm biomass was observed in mast cell deficient rats than in control rats after secondary infection (Fig. 4). This suggests that mast cells have a role in worm biomass control as part of reinfection resistance. No significant difference in worm numbers was seen between control and mast cell deficient rats after secondary infection (Fig. 3); this suggests that mast cells are not involved in worm expulsion. Mucosal mastocytosis plays a role in the protective host response to some nematode infections [21]. However, the difference in timing of tapeworm expulsion and mucosal mastocytosis [11] and the existence of a rat strain with mucosal mastocytosis that does not expel tapeworms [10], suggest that the mast cell has no direct role in the expulsion of *H. diminuta* from rat intestines. On the other hand, the consistent timing of the decrease in worm biomass and increase in mastocytosis in

specific rat strains suggests that the mast cell has some effect on worm biomass of this tapeworm [20]. It has been reported that basophils act in acquired immunity in collaboration with antibodies bound to their Fcε receptors against *Nippostrongylus brasiliensis* infection [21]. A similar mechanism might be present in the protective role of mast cells because these cells express Fcε receptors similarly to basophils. Our experiments using primary and secondary infections by *H. diminuta* in WsRC rats (Fig. 3 and Fig. 4) confirmed that mast cells have a limited effect on worm biomass and no effect on worm expulsion, as previously reported [10, 11, 20]. Overall, our experiments on worm biomass after primary infection with 5 cysticercoids of rats with a WsRC genetic background (Fig. 1) clearly revealed that the mast cell is not a central player in determining the worm biomass of the rat intestine. These findings imply the existence of other effector cells to control carrying capacity. One such candidate is the goblet cell, which is an important effector cell for gut nematode expulsion through mucin production [22]. However, infection of rats with 5 cysticercoids does not cause a significant change in mucin 2 production (the secreted form of small intestinal mucin), protein and gene expression [19]. It is possible that effector cells other than mast cell and goblet cell control the carrying capacity of *H. diminuta* in the rat intestine. We speculate that eosinophils are another candidate because the number of these cells increases rapidly during worm growth, in contrast to mast cells and goblet cells [23].

An increase in worm expulsion and a decrease in worm biomass due to reinfection resistance were clearly observed after secondary infection of F344 rats with *H. diminuta* (Fig. 3 and Fig.4), as previously reported [1, 18]. The reinfection resistance in control WsRC-+/+ rats with normal immunity was considerably weaker than in F344 rats (Fig. 3 and Fig. 4). This suggests that reinfection resistance is influenced by the genetic background of the rat strain, and that F344 rats are high responders and WsRC rats are low responders to reinfection. It is possible that any effect of mast cells in reinfection resistance was masked on

the low responder (WsRC) genetic background.

The life cycle is an important aspect of the relationship between a parasite and its host. Some hymenolepidid species have irregular life cycles, for example, *H. nana* can complete its development from egg to adult worm directly in the mammalian definitive host [24, 25], while *H. microstoma* can develop into mature adults from eggs in the gastrointestinal tracts of immuno-deficient mouse strains such as NMRI-*nu* and NOD-*scid* [26]. We examined the possibility of a direct life cycle in *H. diminuta* in immuno-deficient rats. Adult worm numbers did not change in F344-*rnu* and F344-*xscid* rats until 12 weeks after 5 cysticercoid infection, and no cysticercoids were observed in the intestines of these rats. Furthermore, in a preliminary study, no cysticercoids or adult worms were found in the small intestine of F344-*xscid* rats at 6 weeks after oral administration of 2×10^5 eggs of *H. diminuta.* These results indicate that *H. diminuta* cannot undertake a direct life cycle even in immune-deficient rats, unlike *H. nana* and *H. microstoma*.

One of the advantages of *H. diminuta* as a laboratory model is that it shows comparable host-parasite interactions in rats (definitive host) to those in mice (non-definitive host). However, at present our understanding of the basic relationships between adult *H. diminuta* tapeworms and the intestine of the definitive host (rat) is insufficient. In this study, we demonstrated a major role for host immunity in determining the carrying capacity of *H. diminuta* in rat intestines and a weak role for mast cells on tapeworm biomass; however, the major effector cell that controls worm biomass has still to be identified. Further analyses of the *H. diminuta* infected rat model may reveal the particular characteristics of parasite-host interactions in this non-invasive intestinal large helminth.

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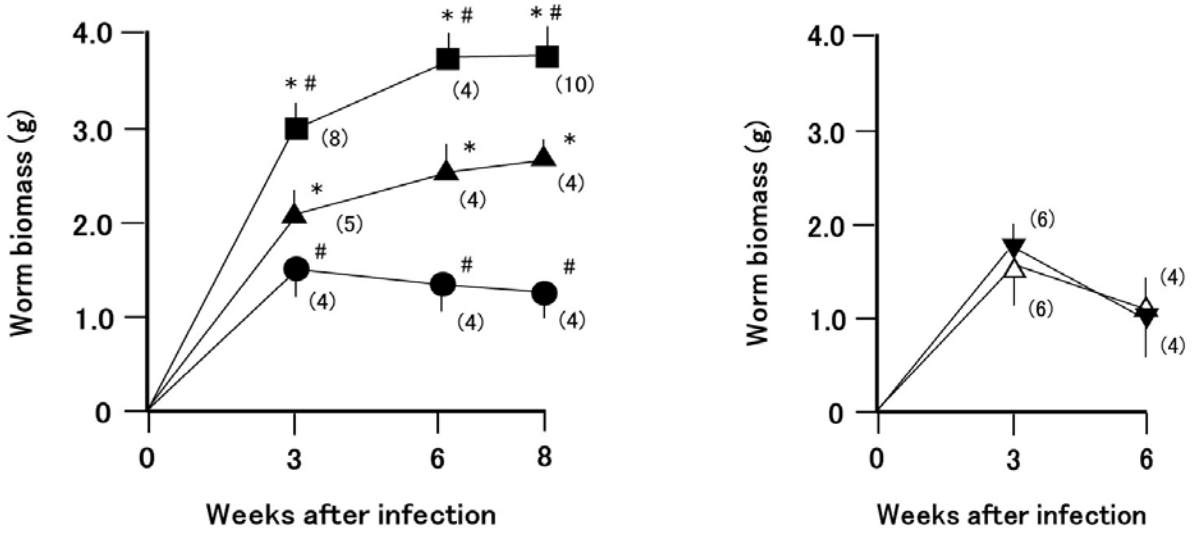
Figure legends

Fig. 1 Effects of host genome mutations on worm biomass after 5 cysticercoid infection of rats with either an F344 (left side) or WsRC (right side) genetic background. The figures in parentheses indicate the number of rats examined. ●, F344; ▲, F344-*rnu*; ■, F344-*xscid*; ∆, WsRC-*Ws/Ws*; ▼, WsRC-+/+. *, significantly different at $P < 0.05$ versus F344 by Bonferroni's test; #, significantly different at $P < 0.05$ versus F344-*rnu* by Bonferroni's test. There were no significant differences among rats on the WsRC genetic background by Student's t-test.

Fig. 2 Comparison of total worm biomass (left side) and mean worm weight (right side) of F344 rats with that in F344-*xscid* rats at 3 weeks after infection with different numbers of cysticercoids. *, significantly different at $P < 0.05$ by Bonferroni's test versus F344 given the same level of cysticercoid infection; #, significantly different at $P < 0.05$ by Bonferroni's test versus infection with 1 cysticercoid of rats with the same strain.

Fig. 3 Comparison of recovered worm numbers between primary (1st) and secondary (2nd) infections at 3 weeks after infection with 5 cysticercoids. Left, F344 rats; right, WsRC rats. *, significantly different at $P < 0.05$ by Bonferroni's test versus 1st infection of rats with the same strain; #, significantly different at $P < 0.05$ by Bonferroni's test versus control rats infected in the same manner.

Fig. 4 Comparisons of worm biomass between primary (1st) and secondary (2nd) infection at 3 weeks after infection with 5 cysticercoids. Left, F344 rats; right, WsRC rats. *, significantly different at $P < 0.05$ by Bonferroni's test versus 1st infection of rats with the same strain; #, significantly different at $P < 0.05$ by Bonferroni's test versus control rats infected in the same manner.


Worm biomass (g)
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3.0
2.0
1.0
0
0
3
6
8
Weeks after infection
* #
(8)
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Worm biomass (g)
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1.0
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Weeks after infection
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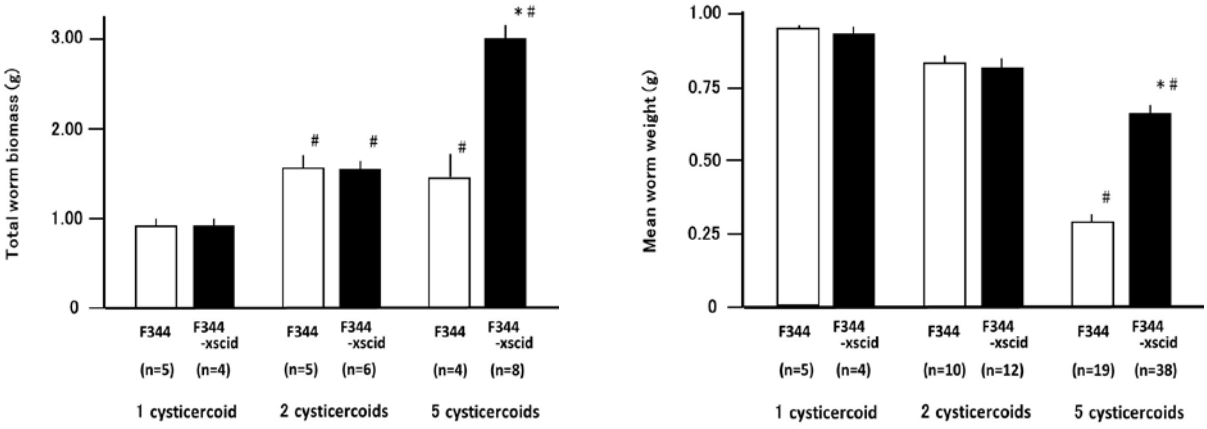
Fig.1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2.00
1.00
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*#
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#
#
F344
F344 -xscid
(n=5)
(n=4)
(n=5)
(n=6)
(n=4)
(n=8)
1 cysticercoid
2 cysticercoids
5 cysticercoids
Mean worm weight (g)
1.00
0.75
0.50
0.25
0
*#
#
(n=5)
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(n=12)
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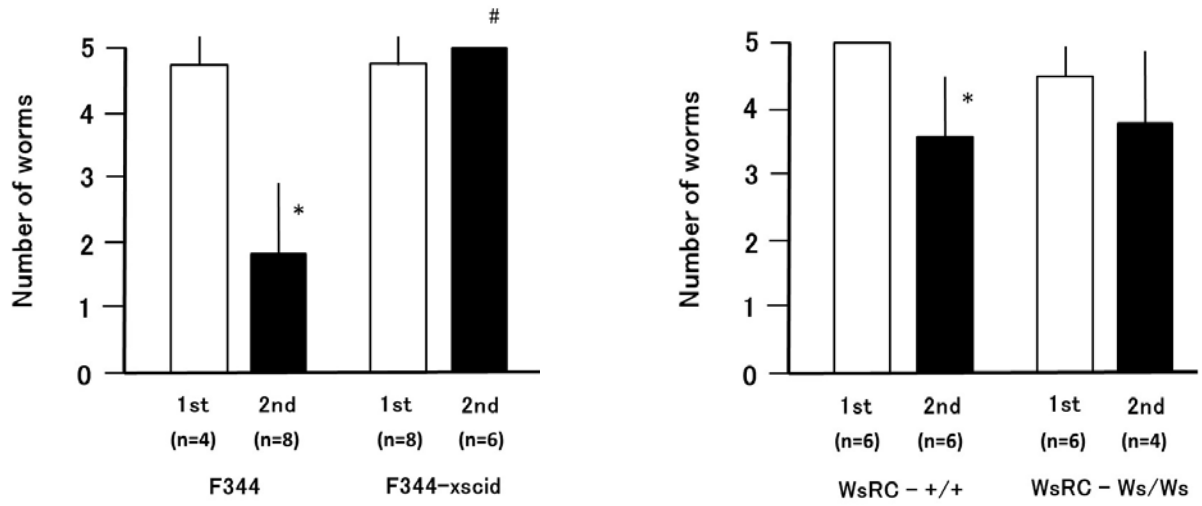
(n=38)
1 cysticercoid
2 cysticercoids
5 cysticercoids

Fig.2


Number of worms
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4
3
2
1
0
*
#
1st
2nd
1st
2nd
(n=4)
(n=8)
(n=8)
(n=6)
F344
F344-xscid
Number of worms
5
4
3
2
1
0
*
1st
2nd
1st
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(n=6)
(n=6)
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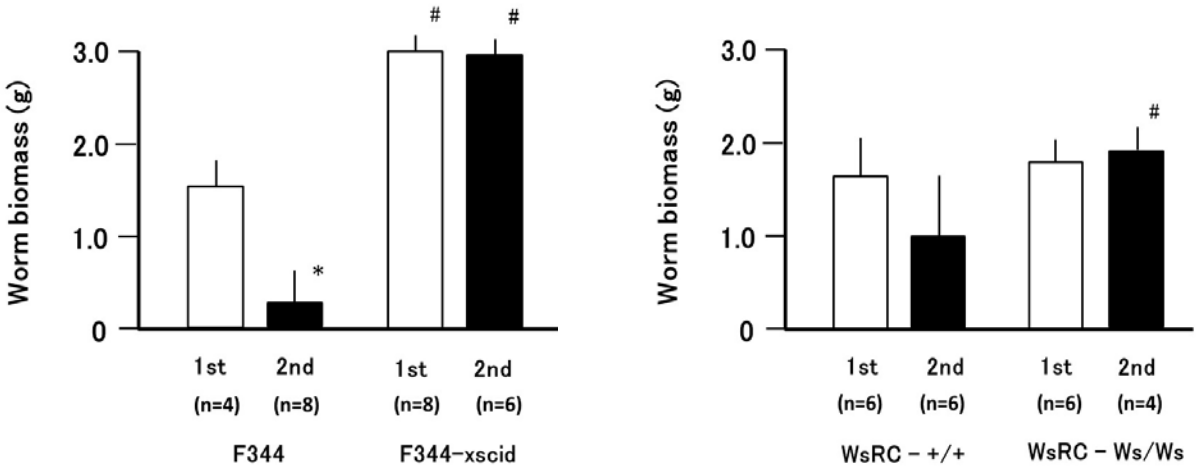
WsRC - +/+
WsRC - Ws/Ws

Fig.3


Worm biomass (g)
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2.0
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1st
2nd
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(n=4)
(n=8)
(n=8)
(n=6)
F344
F344-xscid
Worm biomass (g)
3.0
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1st
2nd
(n=6)
(n=6)
(n=6)
(n=4)
WsRC - +/+
WsRC - Ws/Ws

Fig.4