# Analysis for genetic loci controlling protoscolex development in the *Echinococcus multilocularis* infection using congenic mice

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Abstract

The resistance/susceptibility to *Echinococcus multilocularis* infection in mice is genetically controlled. However, genetic factors responsible for these differences remain unknown. Our previous study in genetic linkage analysis has revealed that there is a significant quantitative trait locus (QTL) for the establishment of cyst (*Emcys1*), and a highly significant QTL for the development of protoscolex of *E. multilocularis* larvae (*Empsc1*), on mouse chromosomes 6 and 1, respectively. The current study aimed to confirm these QTLs and narrow down the critical genetic region that controls resistance/susceptibility to *E. multilocularis* infection by establishing congenic and subcongenic lines from C57BL/6 (B6) and DBA/2 (D2) mice. For protoscolex development phenotype, two congenic lines, B6.D2-*Empsc1* and D2.B6-*Empsc1* were developed, where responsible QTL, *Empsc1* was introgressed from D2 into B6 background and *vice versa*. For cyst establishment phenotype, two congenic lines, B6.D2-*Emcys1* and D2.B6-*Emcys1* were developed, where responsible QTL, *Emcys1* was introgressed from D2 into B6 background and *vice versa.* Because there was no significant difference in cyst establishment between B6.D2-*Emcys1* and D2.B6-*Emcys1* mice after challenge with *E. multilocularis*, it is suggested that the *Emcys1* does not solely control the cyst establishment in mouse liver. However, infection experiments with B6.D2-*Empsc1* and D2.B6-*Empsc1* mice showed a significant difference in protoscolex development in the cyst. It confirms that the *Empsc1* controls phenotype of the protoscolex development in the cyst. Subsequently, two subcongenic lines, B6.D2-*Empsc1.1* and B6.D2-*Empsc1.2* from B6.D2-*Emcys1* and one subcongenic line, D2.B6-*Empsc1.1* from D2.B6-*Empsc1* were developed to narrow down the critical region responsible for protoscolex development. From the results of infection experiments with *E. multilocularis* in these subcongenic mice, it is concluded that a gene responsible for protoscolex development is located between *D1Mit290* (68.1 cM) and *D1Mit511* (97.3 cM).

**Key Words:** *Echinococcus multilocularis*; protoscolex; congenic mouse; quantitative trait locus

## 1. Introduction:

The aim of the current research is to verify genetic loci responsible for controlling the development of alveolar echinococcosis (AE) by using congenic and subcongenic mice. AE is a zoonotic disease caused by the metacestode stage of tapeworm *Echinococcus multilocularis* characterized by a severe hepatic disorder in many parts of the northern hemisphere (Craig et al., 1996). Infiltrating parasitic growth (hematologous spread) can cause severe organ damage. Long term treatment is one of the expansive issues and causing health problems (Kern at al., 2003; Reuter et al., 2000). Mainly rodents and occasionally humans become the intermediate hosts being infected by oral ingestion of eggs excreted from contaminated feces of the definitive host carnivores in the environment. The eggs hatch out into oncospheres in the intestine of intermediate hosts. The oncospheres migrate to the liver by penetrating the intestinal wall and develop to metacestode. Mature metacestodes produce huge numbers of protoscoleces in intermediate hosts. (Gottstein et al., 1995). Mice have been widely used as an experimental model to study the host-parasite interplay in the *E. multilocularis* infection. Matsumoto et al (2010) demonstrated that two inbred strains, C57BL/6 (B6) and DBA/2 (D2) mice, differed markedly in their susceptibility to *E. multilocularis* infection. The oral administration of *E. multilocularis* eggs to D2 mice established a higher number of cysts in the liver as compared to B6 mice. Moreover, a significant number of protoscoleces were observed in the cysts of D2 mice, whereas protoscoleces were completely absent in B6 mice. Additionally, the observation from other studies have supported the results that the susceptibility to *E. multilocularis* infection is genetically controlled both in animals and humans (Nakaya et al., 1997; Hildreth and Granholm, 2003; Vuitton, 2003). Several studies have reported that B6 and D2 mice have susceptibility difference against various infectious diseases (David et al., 1995; Adrianus et al., 2009; Marquis et al., 2009; Simon et al., 2009). The genetic factors responsible for difference in the susceptibility to *E. multilocularis* parasite still remains unknown. Considering the marked difference between B6 and D2 mice in susceptibility to *E. multilocularis* infection, quantitative trait locus (QTL) analysis was conducted in backcrossed progenies from B6 and D2 mice (Nakao et al., 2011). QTL mapping is a promising tool

for the detection of genetic loci that contribute to the determination of differences in phenotypic variation. QTL analysis has revealed a significant QTL, *Emcys1* for the establishment of *E. multilocularis* cysts in the mouse liver, suggesting that it controls the number of cysts in the liver (Nakao et al., 2011). Furthermore, the development of protoscoleces in cysts in the mouse liver were controlled by a distinct highly significant QTL, *Empsc1*, indicating a role of different host factor interplaying with parasites at each developmental stage (Nakao et al., 2011). Lack of information in the interplay between parasite and the intermediate host makes it difficult to focus on certain genes responsible for resistance/susceptibility to *E. multilocularis* infection. It is critically important to address the role of genetic factors to understand the course of infection as well as to get better treatment strategies. To verify that previously identified QTLs are indeed responsible for susceptibility or resistance to *E. multilocularis* infection, making congenic mouse strains is one of the most reliable strategies. Congenic mouse strains are defined as those, in which genetic alteration or mutation is transferred into a standard inbred mouse strain (Markel et al., 1997). Several researchers have published valuable findings using congenic mice that could confirm the responsible genetic regions to address candidate genes in various diseases, including infectious and autoimmune diseases (Shimizu et al., 2007; Marquis et al., 2008; Allen et al., 2006). The purpose of the current study is to verify that previously identified QTLs are indeed responsible for resistance/susceptibility to *E. multilocularis* infection by establishing congenic mouse strains from B6 and D2 mice, where the significant QTLs are introgressed from B6 to D2-genetic background and *vice versa* from D2 to B6-genetic background. Furthermore, subcongenic lines were generated to narrow down the critical region, including QTLs responsible for resistance/susceptibility to *E. multilocularis* infection.

## 2. Materials and methods

### *2.1. Mice*

Specific pathogen-free inbred mice, C57BL/6NSlc (B6) and DBA/2CrSlc (D2) were purchased from Japan SLC (Shizuoka, Japan) to generate congenic and subcongenic lines. For generation of congenic and subcongenic lines, we were adhered to the AAALAC International-accredited program and the Regulation for the Care and Use of Laboratory Animals, Hokkaido University, and animal use protocol was approved by the President of Hokkaido University after the review by the Institutional Animal Care and Use Committee. Parental inbred, congenic, and subcongenic mice were subjected to infection experiments with *E. multilocularis*. Infection experiments were performed in accordance with the regulation of Hokkaido Institute of Public Health and animal use protocol was approved by President of Hokkaido Institute of Public Health after the review by the Ethics Committee of the Institute.

#### *2.1.1. Congenic lines*

For the *Emcys1*, two congenic lines were generated by the introgression of *Emcys1* in chromosome (Chr) 6 from B6 to D2-genetic background and *vice versa* from D2 to B6-genetic background. The (B6 x D2)$F_1$ mice were backcrossed to B6 or D2 parental strain [(B6 x D2)$F_1$ x B6 or (B6 x D2)$F_1$ x D2]. Congenic lines were generated by the speed congenic method (Markel et al., 1997). Briefly, in case of B6-genetic background, backcrossing was repeated up to 5 generations and homozygous founders were established by brother-sister mating. Similarly, for the D2-genetic background, backcrossing was repeated up to 7 generations and homozygous founders were established by brother-sister mating. For the *Empsc1*, two congenic lines were generated according to the same method as for the *Emcys1*-congenic lines, where *Empsc1* in Chr 1 was introgressed from B6 to D2-genetic background and *vice versa* from D2 to B6-genetic background. For both genetic backgrounds (B6 and D2), backcrossing was repeated up to 6 generations and homozygous founders were established by

brother-sister mating. Introgression of chromosomal regions was confirmed by genotyping of microsatellite markers shown in Figs. 1 and 5. Congenic lines were named according to the international nomenclature guidelines and abbreviated as in parenthesis; B6.D2-(*D6Mit188-D6Mit15*)/NSlcHkv (B6.D2-*Emcys1*), D2.B6-(*D6Mit188-D6Mit15*)/CrSlcHkv (D2.B6-*Emcys1*), B6.D2-(*D1Mit191-D1Mit291*)/NSlcHkv (B6.D2-*Empsc1*), and D2.B6-(*D1Mit191-D1Mit291*)/CrSlcHkv (D2.B6-*Empsc1*).

*2.1.2. Subcongenic lines*

Two subcongenic lines were developed from B6.D2-*Empsc1*. B6.D2-*Empsc1* was mated with B6 parental strain to generate heterozygous $F_1$ and then $F_1$ generations were backcrossed to B6 to establish heterozygous subcongenic lines. After that, each line was intercrossed (brother-sister mating) to get homozygous subcongenic lines. Another subcongenic line was developed from D2.B6-*Empsc1* according to the same method as subcongenic line from B6.D2-*Empsc1*. D2.B6-*Empsc1* was mated with D2 parental strain to generate $F_1$ heterozygous subcongenic mice and homozygous subcongenic line from D2.B6-*Empsc1* was established by brother-sister mating. Introgression of chromosomal regions was confirmed by genotyping of microsatellite markers as shown in Fig. 1. Subcongenic lines were named according to the international nomenclature guidelines and abbreviated as in parenthesis; B6.D2-(*D1Mit191-D1Mit290*)/NSlcHkv (B6.D2-*Empsc1.1*), B6.D2-(*D1Mit201-D1Mit291*)/NSlcHkv (B6.D2-*Empsc1.2*), and D2.B6-(*D1Mit191-D1Mit14*)/CrSlcHkv (D2.B6-*Empsc1.1*).

*2.2 Microsatellite markers and genotyping*

A total of 134 microsatellite markers (Supplementary Table 1) were selected from the database in the Mouse Genome Informatics (MGI), The Jackson laboratory, ME, USA (http://www.informatics.jax.org/) to generate congenic and subcongenic lines. Twenty-eight

161 microsatellite markers (Figs. 1 and 5) were used to confirm the introgressed regions for cyst
162 establishment and protoscolex development in congenic and subcongenic mice. Briefly, a piece of
163 ear-punched tissue was collected and genomic DNA was extracted by incubating samples at 54 °C for
164 3 h in 0.5 ml of lysis buffer [10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10 mM
165 ethylenediaminetetraacetic acid (EDTA) and 1% sodium dodecyl sulfate (SDS)] with 5 µl of 10
166 mg/ml proteinase K. After genomic DNA extraction the touchdown PCR was performed with *Taq*
167 DNA polymerase (Ampliqon A/S, Odense M, Denmark) as follows; denaturing at 95 °C for 1 min,
168 followed by 10 cycles of denaturing at 95 °C for 30 sec, primer annealing at 65 °C for 30 sec (-2 °C in
169 2 cycles), and extension at 72 °C for 30 sec, and then, 35 cycles of denaturing at 95 °C for 30 sec,
170 primer annealing at 55 °C for 30 sec, and extension at 72 °C for 30 sec, and final extension at 72 °C
171 for 1 min, using a T100$^{TM}$ thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). The
172 amplified samples were electrophoresed in 12% polyacrylamide gels in TBE buffer (89 mM Tris, 89
173 mM boric acid, and 2 mM EDTA), and stained with ethidium bromide for the visualization under a
174 UV light. The genotype was identified by the size of the PCR product.
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176 *2.3 Infection experiments and phenotype assessment*
177
178 The infection experiments were performed according to Nakao et al., (2011). Briefly, *E.*
179 *multilocularis* eggs were collected from feces of the infected dogs. Experimental infection was
180 conducted in parental inbred strains (B6 and D2), congenic, and subcongenic mice by oral
181 administration of *E. multilocularis* eggs. For the assessment of cyst establishment, mice were infected
182 with 200 eggs, sacrificed, and necropsied at 4 weeks after infection. Mouse liver was cut into small
183 slices with approximately 0.5-mm thickness and the total number of cysts was counted in each liver.
184 For the assessment of protoscolex development in the cyst, mice were infected with 2,000 eggs,
185 sacrificed, and necropsied at 16 weeks after infection, because larger number of eggs and longer period
186 were needed to assess protoscolex development in the cyst. Livers were collected, parasitic cysts were
187 dissected from the liver, and total weight of the cysts was measured. A part of the cysts (1-2 g) was

minced, passed through a 125-μm sieve, and washed repeatedly with saline. The number of mature protoscoleces was counted under a stereoscopic microscope and expressed as number/gram of cysts. Moreover, protoscoleces were counted in histopathological sections of liver to confirm the protoscolex development in cysts.

*2.4. Histopathology*

Liver tissues were fixed in 10% formalin in phosphate-buffered saline (pH 7.4). The specimen was dehydrated in consecutive stages with increasing concentrations of alcohol and cleaned by xylene. Finally, liver samples were processed for paraffin embedding and cut into 2-μm-thick sections. Tissue sections were stained with haematoxylin and eosin for microscopic examinations with an All-in-One Fluorescence Microscope BZ-X710 (Keyence, Osaka, Japan) to confirm protoscolex development in the liver section.

*2.5. Statistical analysis*

All data were subjected to non-parametric test, Mann-Whitney *U*-test to compare between two groups and one-way ANOVA with Bonferroni post-hoc analysis for more than two groups. Statistical analyses were performed using a GraphPad Prism 5 software version 5 for windows (GraphPad software Inc., San Diego, CA, USA). $P < 0.05$ was considered to be significant.

## 3. Results

*3.1 Confirmation of Empsc1 using congenic mice*

In congenic mice, B6.D2-*Empsc1* and D2.B6-*Empsc1*, introgressed chromosomal region (*D1Mit7* to *D1Mit511*) was confirmed by genotyping microsatellite markers (Fig. 1). This region covered

highly significant region of the QTL peak detected in the previous QTL analysis (Nakao et al., 2011). Other chromosomes except for Chr 1 were confirmed to be recipient-genetic background by genotyping microsatellite markers located in other chromosomes as shown in Supplementary Table 1. To confirm the *Empsc1* for protoscolex development, B6.D2-*Empsc1* and D2.B6-*Empsc1* mice were subjected to examination at 16 weeks after oral administration of 2,000 eggs of *E. multilocularis*. The number of mature protoscoleces in the cysts was calculated in B6.D2-*Empsc1* and D2.B6-*Empsc1* as well as B6 and D2 mice (parental strains). The number of protoscolex in the cyst of B6.D2-*Empsc1* was 28,285 ± 15,819, comparable to that of D2 (28,255 ± 5,124), and significantly greater than that in B6 mice, which was completely absent (Fig. 2A). On the other hand, the number of protoscolex in the cyst of D2.B6-*Empsc1* mice was 5,128 ± 2,902 and significantly less than that of D2 control mice (239,709 ± 65,461) (Fig. 2B). These results indicate that the *Empsc1* is definitely located between *D1Mit7* and *D1Mit511* and contributes to the difference in protoscolex development between B6 and D2 mice.

*3.2 Narrowing the critical genetic region*

To narrow down the length of critical region, three subcongenic lines were developed from congenic mice, B6.D2-*Empsc1* and D2.B6-*Empsc1*. Genotyping of microsatellite markers revealed that two lines of B6.D2-subcongenic mice, B6.D2-*Empsc1.1* and B6.D2-*Empsc1.2*, were introgressed D2-derived chromosomal region between *D1Mit7* and *D1Mit399* (38.6 cM and 70.3 cM) and *D1Mit496* and *D1Mit511* (63.1 cM and 97.3 cM), respectively (Fig.1). On the other hand, D2.B6-subcongenic line, D2.B6-*Empsc1.1,* was confirmed to be introgressed B6-derived chromosomal region between *D1Mit7* and *D1Mit290* (38.6 cM and 68.1 cM) in the D2-genetic background (Fig.1).

These three lines of subcongenic mice were challenged with *E. multilocularis* infection and protoscoleces in the cysts were counted under a light microscope. The number of protoscolex in the cyst of B6.D2-*Empsc1.2* and D2.B6-*Empsc1.1* were 22,700 ± 7,651 and 55,660 ± 8,588, respectively, indicating a significant development of protoscoleces as well as D2 mice (104,367 ± 48,349), whereas

B6.D2-*Empsc1.1* and B6 mice did not develop protoscoleces (Fig. 3). The number of B6.D2-*Empsc1.2* and D2.B6-*Empsc1.1* was significantly different, although protoscoleces were significantly developed in these two subcongenic strains. This difference may be due to the difference in genetic background. Thus, some genetic factors contributing to the protoscolex development may be present in the D2 genetic background besides the introgressed regions. Alternatively, another genetic factor affecting the protoscolex development may exist in the D2-derived region existing in B6.D2-*Empsc1.2* but not in D2.B6-*Empsc1.1*, that is a region between *D1Mit496* and *D1Mit290*. The third possibility is retaining some fragmental regions derived from the donor strain in congenic strains that affect protoscolex development. Nonetheless, these results conclude that the *Empsc1* is located in the critical region between *D1Mit290* and *D1Mit511* (68.1 cM and 97.3 cM) and is secured in the introgressed region of B6.D2-*Empsc1.2* (Fig.1).

*3.3 Confirmation of protoscolex in subcongenic mice with histopathological analysis*

Protoscolex development in subcongenic mice was confirmed by histopathological analysis of liver sections. Protoscoleces in the cyst of B6.D2-*Empsc1.2* were developed as well as in D2 mice, whereas B6.D2-*Empsc1.1* mice did not develop any protoscolex as seen in B6 mice (Fig. 4A). The mean number of protoscoleces in the cyst of D2 and B6.D2-*Empsc1.2* was $6.2 \pm 1.1$ and $7.8 \pm 1.9$, respectively, and statistically different from that of B6 and B6.D2-*Empsc1.1* (Fig. 4B). These results are consistent with results from the actual counting under a light microscope.

*3.4 Congenic lines for cyst establishment*

Congenic lines, B6.D2.-*Emcys1* and D2.B6-*Emcys1*, for cyst establishment phenotype were confirmed introgressed region by genotyping microsatellite markers (Fig. 5). Both congenic lines were replaced chromosomal region between *D6Mit188* and *D6Mit15* with donor haplotype, in which *Emcys1* locus could be included based on the QTL analysis data in the previous paper (Nakao et al.,

2011). Other chromosomes except for Chr 6 were confirmed to be recipient-genetic background by genotyping of microsatellite markers locating in other chromosomes as shown in Supplementary Table 1. These congenic mice were then challenged with *E. multilocularis* infection and investigated cyst establishment (Fig. 6). The number of cysts established in liver of B6.D2.-*Emcys1* and D2.B6-*Emcys1* congenic mice was 7.5 ± 2.3 and 35.5 ± 5.9, respectively, and was not different from that of each parental strain, B6 (13.2 ± 3.2) and D2 (41.2 ± 4.7), indicating that the replacement of *Emcys1* locus with the donor genotype did not alter the recipient phenotype. The reason for the ineffectiveness of the *Emcys1* introgression is unknown; however, our result indicates that the number of cysts is not controlled by the *Emcys1* alone.

## 4. Discussion

A previous investigation showed that susceptibility or resistance to *E. multilocularis* infection was genetically controlled and D2 mice were more susceptible to infection than B6 mice (Matsumoto et al., 2010). Further, another study identified a significant QTL, *Emcys1* in Chr 6 and a highly significant QTL, *Empsc1* in Chr 1 as responsible for cyst establishment and protoscolex development, respectively (Nakao et al., 2011).

In this study, congenic lines, B6.D2-*Empsc1* and D2.B6-*Empsc1*, were established from B6 and D2 parental strains and it was confirmed that the *Empsc1* is responsible for the difference in the protoscolex development. As 834 protein-coding genes are present in the introgressed chromosomal region between *D1Mit7* and *D1Mit511*, narrowing down the introgressed region could lead to the identification of candidate genes responsible for protoscolex development. Therefore, subcongenic lines, B6.D2-*Empsc1.1* and B6.D2-*Empsc1.2*, were generated from B6.D2-*Empsc1* and D2.B6-*Empsc1.1* line was generated from D2.B6-*Empsc1* (Fig.1). These subcongenic mice were challenged with *E. multilocularis* infection and we found protoscolex development in B6.D2-*Empsc1.2* mice, but not in B6.D2-*Empsc1.1*. On the other hand, D2.B6-*Empsc1.1* mice showed protoscolex development as seen in D2 mice. From these results, we could narrow down a critical region between *D1Mit290*

and *D1Mit511*, in which a gene responsible for protoscolex development must be located. Based on MGI database, there are still 331 protein-coding genes located in the critical region, among which several candidate genes such as those relating to the immune response or inflammation are listed in Table 1. However, at this point it is difficult to address responsible gene(s) until functional verification of gene(s) controlling the protoscolex development is carried out. Yang et al (2006) reported that host genetic factors could contribute to the susceptibility of humans to *E. multilocularis*. Moreover, some genes showed quantitative genetic variation in mice as well as in humans (Korstanje et al. 2004; Hellebrandtet et al., 2005). Many researchers have proposed that the interplay between parasites and hosts has been developed by an evolutionarily conserved signaling system, specifically the interaction between parasitic receptors and host-derived molecules (Gelmedin et al., 2008; Brehm, 2010). Our results from this study could facilitate the identification of candidate gene(s) involving this signaling pathway for protoscolex development in both mouse and human, leading to the implementation of the most effective control strategy for AE. Other researchers have successfully addressed candidate genes responsible for some genetic phenotypes with the linkage analysis using congenic and subcongenic lines (Pelletier et al., 2016; Kanagaratham et al., 2014) and gene expression profiling in subcongenic mouse lines (Sander et al., 2007; Rennie et al., 2008; Ahn et al., 2010; Stark et al., 2010). Therefore, we have started to produce backcrosses from B6.D2-*Empsc1.2* to identify responsible gene(s) with genetic linkage analysis.

In case of cyst establishment phenotype, two congenic lines, B6.D2-*Emcys1* and D2.B6-*Emcys1*, were generated from parental strains B6 and D2, respectively. The results from the current study could not confirm that the genetic region between *D6Mit188* and *D6Mit15* was responsible for the cyst establishment in the mouse liver (Fig. 6). This result suggests that other genetic factors may be involved in the cyst establishment of *E. multilocularis* in the mouse liver.

In conclusion, a QTL *Empsc1* for protoscolex development of *E. multilocularis* was confirmed by the generation of congenic lines. Moreover, we could narrow down the critical genetic region to the length of 34.2 Mb between *D1Mit290* and *D1Mit511* and secured candidate gene(s) in the introgressed region of B6.D2-*Empsc1.2*. These findings will lead to the identification of the candidate

gene(s) for protoscolex development of *E. multilocularis*. Identification of candidate gene(s) will enrich the scientific knowledge of this zoonotic disease echinococcosis and would lead to the development of new and effective drugs against echinococcosis.

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**Legends to figures**

**Fig. 1.** Schematic diagram of Chr1 in congenic and subcongenic mice for *Empsc1*. Shaded, open, and black columns indicate B6-derived, D2-derived, and unknown chromosomal portions, respectively.

**Fig. 2.** Protoscolex development in the liver of B6, D2, and congenic mice for *Empsc1*. The number of protoscolex in cystic lesions of the liver was counted and expressed as number of protoscolex per 1 gram of cystic lesion. (A) Data represent the mean ± SEM for B6 (n=4), D2 (n=4), and B6.D2-*Empsc1* (n=5). (B) Data represent the mean ± SEM for D2 (n=9) and D2.B6-*Empsc1* (n=3). Data are representative of two independent experiments. ** and * indicate $P < 0.01$ and $P < 0.05$, respectively.

**Fig. 3.** Protoscolex development in the liver of B6, D2, and subcongenic mice for *Empsc1*. The number of protoscolex in cystic lesions of the liver was counted and expressed as number of protoscolex per 1 gram of cystic lesion. Data represent the mean ± SEM for B6 (n=3), D2 (n=3) and subcongenic mice for *Empsc1*, B6.D2-*Empsc1.1* (n=3), B6.D2-*Empsc1.2* (n=3), and D2.B6-*Empsc1.1* (n=5). * indicates $P < 0.05$.

**Fig. 4.** (A) Photographs of liver sections in B6, D2, and subcongenic mice for *Empsc1*. Arrows indicate protoscolecces. (B) Protoscolex counts in liver sections in B6 (n=6), D2 (n=6), B6.D2-*Empsc1.1* (n=6), and B6.D2-*Empsc1.2* (n=8). The number of protoscolex was counted in 10 cysts selected randomly in each mouse liver and the mean value was calculated in each mouse. Data represent the mean ± SEM. ** and * indicate $P < 0.01$ and $P < 0.05$, respectively.

**Fig. 5.** Schematic diagram of Chr 6 in congenic mice for *Emcys1*; B6.D2- *Emcys1* and D2.B6- *Emcys1*. Shaded, open, and black columns indicate B6-derived, D2-derived, and unknown chromosomal portions, respectively.

455 **Fig. 6.** Cyst establishment in the liver of B6 (n=6), D2 (n=6), and congenic mice for *Emcys1*; B6.D2-
456 *Emcys1* (n=5) and D2.B6- *Emcys1* (n=6). The data represent mean ± SEM. Data are representative of
457 two independent experiments. ** indicates $P < 0.01$.