Meriones meridianus and Lagurus lagurus as Alternative Definitive Hosts of Echinococcus multilocularis and E. granulosus

Yuzaburo OKU1, Jiao WEI2, June-Jie CHAI2, Irsayil OSMAN3, Jian WEI2, Li-fu LIAO2, Mitsuhiko ASAKAWA3, Katsuro HAGIWARA3, Kimio KOBAYASHI4, and Mamor ITO4

1Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan,
2Xinjiang Institute for Endemic Diseases Control and Research, Urumqi, Xinjiang 83002, China,
3School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, 069-8501,
4Central Institute for Experimental Animal, Kawasaki 216-0001, Japan

Abstract: The utilization of Meriones meridianus and Lagurus lagurus as alternative definitive hosts for Echinococcus multilocularis and E. granulosus was investigated. Tapeworm stage development of E. multilocularis was observed and their recovery rate was determined in the small intestine of M. meridianus and L. lagurus. These were compared with those in golden hamsters, which are known as alternative definitive hosts. The animals were treated with PTBA (prednisolone tertiary butylacetate) and PA (prednisolone acetate), after which M. meridianus showed the highest recovery rate, whereas L. lagurus had few or no worms. The recovery rate of worms from golden hamsters was between those of M. meridianus and L. lagurus. On day 20 post-infection, developing tapeworms with mature segments were collected from M. meridianus treated with PA. The worms were mostly from the proximal and medial small intestine. The three species of animals infected orally with E. granulosus were divided into two groups, PTBA-treated and non-treated control groups. PTBA promoted/enhanced the recovery rate of the worms until 5 days, but none or only a few worms were found in PTBA treated animals thereafter. The highest recovery rate was obtained from M. meridianus treated with PTBA on day 5 post-infection. Some worm developments were observed on days 5 and 10 post-infection. These results demonstrate that M. meridianus could be useful as an alternative definitive host of Echinococcus.

Key words: alternative definitive host, Echinococcus, golden hamster, Lagurus lagurus, Meriones meridianus

Introduction

Echinococcus multilocularis and Echinococcus granulosus are important zoonotic cestodes in the world, and foxes and dogs are natural definitive hosts, in which the parasites become adult tapeworms with eggs [2]. In definitive hosts, the inoculated protoscoleces (final larval stage in the intermediate host) of Echinococcus,
establish themselves on mucosal surfaces of the small intestine with their scolex after evagination. The parasites form segments, and develop to tapeworm stage with mature segments containing testes and ovary. After fertilization, they produce eggs in the uterus of the last segment, which then detached from the remaining part of body. The detached segments are excreted in the feces of the definitive hosts with their eggs [20]. The prepatent period (period before egg excretion) is 26 days after infection for *E. multilocularis* [21].

Generally, parasites cannot establish themselves in animals other than their hosts. Rodents are generally intermediate hosts, not definitive hosts of *Echinococcus* spp. and *Taenia* spp. in natural environments [15].

Early works suggested the possibility that rodents might be used as alternative definitive hosts for some taeniid cestodes of humans and canids [9, 22]. Cortisone and prednisolone treatment of rodent hosts facilitated the survival, sexual maturation and fertilization of the cestode [11, 19]. We developed alternative definitive host models for *Echinococcus multilocularis*, *Taenia saginata*, *Taenia crassiceps* and *Echinococcus vogeli* [5–8, 13]. *Echinococcus multilocularis*, *Taenia crassiceps* and *Echinococcus vogeli* produced eggs in these rodent alternative definitive hosts [5, 6, 8, 13].

Golden hamsters (*Mesocricetus auratus*) and Mongolian gerbils (*Meriones unguiculatus*) are useful animals for this purpose [14, 18]. The maintenance of dogs, the natural definitive host of *E. multilocularis* and *E. granulosus*, with experimental infection, presents a potential risk for laboratory workers even with special facilities for safe maintenance and handling. Many dogs cannot be used for simultaneous experiments in such facilities. While alternative definitive hosts are needed, the recovery rate of the worm from the small intestine of golden hamsters and Mongolian gerbils is not as high as from the natural definitive hosts, and marked variations have occurred. A new alternative definitive host animal is needed for reproducible experiments and the production of many adult cestodes.

The aim of this study was to find a more susceptible alternative definitive host for *E. multilocularis* and *E. granulosus*. *Lagurus lagurus* and *Meriones meridianus*, which are endemic to Xinjiang, China, were studied as alternative definitive hosts and golden hamsters were used as standard animals. In the present study, the establishment and development of worms in the early phase of infection were studied in the search for new alternative definitive hosts. *M. meridianus* is closely related to the Mongolian gerbil, *M. unguiculatus*.

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**Materials and Methods**

**Experimental Infection with *E. multilocularis***

Thirty *M. meridianus* (ages of these animals were not determined, males and females were mixed) captured in Tulufan, 34 *L. lagurus* and 28 golden hamsters (6 to 8 weeks old, males and females), were used for experimental infection with *E. multilocularis*. These animals were reared and housed in the animal room of Xinjiang Institute for Endemic Diseases Control and Research, Urumqi under conventional conditions and were allowed access to a standard diet and water. Golden hamsters were used as standard animals for alternative definitive hosts. Each *M. meridianus* and *L. lagurus* was kept in individual cages. Generally, steroid treatments promote the susceptibility of animals to parasites. PTBA (prednisolone tertiary butylacetate) has been used in previous experiments on *Echinococcus* and *Taenia* [5, 6, 13, 19]. The animals were divided into two groups: the PTBA (Banyu) treated group, and the PA (prednisolone acetate, Shanghai 9th pharmaceutical factory) treated group. PTBA was injected subcutaneously at 3 mg/head into *L. lagurus* (average body weight, 23.8 g), 5 mg/head into *M. meridianus* (average body weight, 50.3 g), and 5 mg/head into golden hamsters (average body weight, 67.1 g) on days −6, −4, −2, 0 and every other day after infection until necropsy. PA was injected subcutaneously on alternate days after infection until necropsy. Doses of PA were 12.5 mg/head for *L. lagurus* and, 25 mg/head for *M. meridianus* and golden hamsters. Body weight of all animals was determined on −6 DPI (days postinfection) and at necropsy as an indicator of the effects of PTBA and PA.

**Experimental Infection with *E. granulosus***

Thirty-seven golden hamsters, 15 *L. lagurus* and 23 *M. meridianus* (males and females) were used for experimental infection with *E. granulosus*. Animals were 6 to 8 weeks old at the beginning of the study. They were divided into two groups: a PTBA treated group and non-treated group. Twenty hamsters, 11 *L. lagurus* and 14 *M. meridianus* were injected subcutaneously.
with PTBA on days -6, -4, -2, 0 and alternate days after infection until necropsy. PTBA doses were 1.2 mg of PTBA/head for *L. lagurus* (average body weight, 20 g), 2.5 mg/head for *M. meridianus* (average body weight, 40 g) and 5.0 mg/head for golden hamster (average body weight, 80 g). Other animals were not treated with any drugs. All animals were weighed on -6 DPI and at necropsy to assess the effects of PTBA.

**Parasites**

The cyst mass of *E. multilocularis* (Hokkaido isolate) was obtained from a cotton rat experimentally infected with 50 eggs, 8 months previously. The cyst mass for protoscolecis inoculation was kept in medium for 3 days with ice before use. Each animal was orally administered approximately 10,000 protoscolecis in 0.3 ml suspension of the cyst mass under ether anesthesia.

The protoscolecis of *E. granulosus* were obtained from the livers of slaughtered sheep in Xinjiang. Each animal was orally administered approximately 10,000 protoscolecis under ether anesthesia.

**Necropsy and parasitological examination**

The small intestines were removed at necropsy, and divided into 3 parts (proximal, middle and distal). Intestinal contents and mucous samples were scraped off each part. All samples were pressed between two glass plates, and observed by microscopy.

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**Results**

**Experimental Infection of *E. multilocularis***

While golden hamsters showed no weight reduction, the weight of *L. lagurus* and *M. meridianus* was reduced after treatment with PTBA and PA until 1 DPI, increased until 9 DPI, and decreased thereafter. A correlation between the recovery rate of worms and weight loss was not observed. *M. meridianus* showed the highest recovery rate among the three species of animals, while few worms (<2 worms) were obtained from *L. lagurus* during the experiment (1–20 DPI) (Figs. 1–3). The recovery rate of worms from golden hamsters was between those of *M. meridianus* and *L. lagurus*. PA had superior effects on the recovery rate of worms in *M. meridianus*. On 20 DPI, developing tapeworms (mean number 212 ± SD 380) with mature segments were collected from the small intestine of *M. meridianus* treated with PA. The worms were found mostly in the proximal and medial small intestine (Fig. 4).

**Experimental Infection of *E. granulosus***

No *E. granulosus* were found in the non-treated *L. lagurus*, and none or few worms were found in PTBA treated *L. lagurus* on 1–5 DPI (Table 1). A few worms (<82) were found in golden hamsters treated or not treated with PTBA, except for 1 hamster (623 worms...
were recovered; almost all of the worms were not evaginated) on 1 DPI. After 15 DPI no worms were found in golden hamsters (Table 2). While PTBA promoted/enhanced the establishment of worms until 5 DPI, none or few worms (<13 worms) were found in the small intestines of the PTBA treated _M. meridianus_ thereafter. The highest recovery rate (16.7%) on 5 DPI was obtained from a _M. meridianus_ treated with PTBA (Table 3). Some developments of the worm were observed on 5 and 10 DPI, when the parasites were fewer.

**Discussion**

In the present study protoscoleces of _E. granulosus_ did not become established in any of the animals for a long time (<10 DPI). In the experimental infection with _E. multilocularis_, PA showed superior effects in _M. meridianus_. PA might be useful in _E. granulosus_ experimental infection, although the PA treatment has not been done. Further study is needed to establish alternative definitive hosts for _E. granulosus_.

_Leishmania infantum_ which causes a visceral type of Leishmaniasis in humans, is known to grow rapidly in _L. lagurus_, thus this animal has been used for studies on Leishmaniasis in China [1, 3]. _L. lagurus_ were not susceptible, however, to oral infection of protoscoleces of _E. multilocularis_ and _E. granulosus_. _Lagurus lagurus_ has been reported as an intermediate host of _E. multilocularis_ in USSR [12], and _Lagurus curtatus_ has the same role in Alaska [15]. In the present study we could not show the utilization of _L. lagurus_ as an alternative definitive host for _Echinococcus_.

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**Fig. 3.** Number of recovered worms from the small intestine of _Meriones meridianus_ treated with PTBA (●) and PA (○) (n=4; except 20 DPI, n=10). *Oral inoculation with 10,000 protoscoleces of _Echinococcus multilocularis_.

**Fig. 4.** Distribution of _Echinococcus multilocularis_ in the small intestine of the PTBA- and PA-treated _Meriones meridianus_ (Mean number and SD). *Oral inoculation with 10,000 protoscoleces.
Table 1. Number of *E. granulosus* recovered from *Lagurus lagurus*

<table>
<thead>
<tr>
<th>DPI</th>
<th>PTBA-treated group</th>
<th>Non-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1, 1</td>
<td>0</td>
</tr>
<tr>
<td>3²)</td>
<td>0, 1, 7, 15, 16</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0, 0, 1, 1</td>
<td>0, 0</td>
</tr>
</tbody>
</table>

²) All worms evaginated after 3 DPI.

Table 2. Number of *E. granulosus* recovered from golden hamsters

<table>
<thead>
<tr>
<th>DPI</th>
<th>PTBA-treated group</th>
<th>Non-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0, 623</td>
<td>0, 0, 5</td>
</tr>
<tr>
<td>3²)</td>
<td>0, 1, 82</td>
<td>0, 1</td>
</tr>
<tr>
<td>5</td>
<td>0, 0, 0, 1, 3, 6, 16</td>
<td>0, 0, 0.7</td>
</tr>
<tr>
<td>15</td>
<td>0, 0, 0, 0, 0</td>
<td>0, 0, 0, 0, 0, 0</td>
</tr>
</tbody>
</table>

²) Most of worms did not evaginate. b) All worms recovered had evaginated by 3 DPI.

The recovery rate of *E. multilocularis* from the golden hamsters was lower on 5–10 DPI than that in a previous study. Sato and Kamiya (1990) reported that the recovery rate on 29 DPI was 7% in hamsters treated from 14 DPI with PTBA. The activity of protoscoleces used in the present experiment might be not ideal because the cyst mass for inoculation of protoscoleces had been kept in medium for 3 days before use. However, the morphological features of protoscoleces used were normal at the time of oral infection, but it is possible that infectivity and ability of the protoscoleces to survive in the alimentary tract were reduced.

*M. meridianus* are pests and common rodents in furrows of Xinjiang as *M. erythraeus* [10]. *M. meridianus* were found to be natural intermediate hosts of *E. multilocularis* in the USSR [4]. *M. meridianus* have been used as experimental intermediate hosts of *E. multilocularis* and *E. granulosus* [16, 17]. They showed higher susceptibility to the larval stage of the parasites than mice. In the present experiment, *M. meridianus* was used as the alternative definitive host. To date, only two animals, golden hamsters and Mongolian gerbils, have been reported as alternative definitive hosts of *E. multilocularis*. In this study, *M. meridianus* showed a higher recovery rate at the tapeworm stage of *E. multilocularis* than golden hamsters (1 to 20 DPI).

Table 3. Number of *E. granulosus* recovered from *Meriones meridianus*

<table>
<thead>
<tr>
<th>DPI</th>
<th>PTBA-treated group</th>
<th>Non-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1, 56, 309, 352</td>
<td>0</td>
</tr>
<tr>
<td>3⁵)</td>
<td>150, 436</td>
<td>0, 2, 9</td>
</tr>
<tr>
<td>5⁵)</td>
<td>0, 183, 1669</td>
<td>0, 0</td>
</tr>
<tr>
<td>10</td>
<td>0, 0, 13</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>0, 0</td>
<td>0</td>
</tr>
</tbody>
</table>

⁵) Half of the worms recovered evaginated. ⁶) All worms recovered had evaginated by 3 DPI. ⁷) The worms became thin.

The results of the present study suggest that *M. meridianus* might be a better alternative definitive host than golden hamster. In Mongolian gerbils and golden hamsters, *E. multilocularis* develop and produce eggs [5, 6]. In the present study long-term observations were not performed, and the reproductive ability of *E. multilocularis* in *M. meridianus* was not determined. Further studies are needed.

**Acknowledgments**

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**References**


