



# HOKKAIDO UNIVERSITY

Title	Improvement of the alternative definitive host model for <i>Echinococcus multilocularis</i> and its application for <i>E. vogeli</i>
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infectious to chickens. The finding of viral receptors should be a key role to define not only the virus-cell interactions but also the oncogenicity of MDV 1. Although different in infectivities to T cell subsets among different MDV strains, the candidate for the viral receptor for those MDV strains appears to be heparan sulfate, at least in the case of the cell-free condition. These results may help to provide a basis for elucidating the functions of various MDV glycoproteins that are essential for the MDV entry to host cells.

Which viral factors are responsible for different oncogenic potentials and for different infectivities among MDV strains? In an attempt to determine those factors, changes in the viral genome were analyzed between oncogenic and non-oncogenic attenuated MDV 1. A novel change in the *meq* gene, which is a candidate for the MDV oncogene, has been found in the attenuated MDV 1.

The *meq* gene of the attenuated MDV 1 was disrupted by the insertion of the 178-bp sequence. These observations suggest that this change could be responsible for differences in not only oncogenic potentials but also infectivities to T cell subsets of MDV 1 strains.

Complicated molecular events are undertaken during the MDV entry to target CD4<sup>+</sup> T cells in chickens, resulting in the variations in infectivities of MDV strains. To define the protection mechanism by MD vaccines, it would be necessary to search both viral ligands which can interact with cell surface heparan sulfate and other cellular receptors. Thus, the clarification of both mechanisms of anti-viral effects by MD vaccines and the molecular characteristics of attenuated MDV 1, especially the function of the elongated *meq* gene, could provide many useful information to develop immunotherapy against not only MDV, but also other virus-induced tumors.

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### Improvement of the alternative definitive host model for *Echinococcus multilocularis* and its application for *E. vogeli*

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The alternative definitive host model for *Echinococcus multilocularis* using laboratory rodents was established and enables us to perform experimental infections of the tape-worm stage with minimum facilities for bio-safety.

In chapter I, improvement of the alterna-

tive definitive host model for *E. multilocularis* was conducted by investigating various drug treatments and by using different host strains and protoscolex isolates. Prednisolone tertiary-butylacetate (PTBA) has been used in the present alternative definitive host model for *E. multilocularis* using Mongolian

gerbil. First, the effect of treatments with other drugs; glucocorticoid, immunosuppressants and non steroid anti-inflammatory drugs, on the number of worms recovered from the small intestine after protoscoleces administration to gerbils were compared. Results showed that subcutaneous and intraperitoneal administration of PTBA were most effective on the establishment of *E. multilocularis*. Second, the doses of PTBA administration were evaluated. A positive correlation was observed between the doses of PTBA treatment and the number of worms recovered from Mongolian gerbils. Third, periods of PTBA treatment were evaluated. Longer treatment with PTBA before infection resulted in the higher number of worms recovered and continuous PTBA treatment after infection contributed to the growth of the parasites. Fourth, the gerbils raised in Hokkaido University (HU strain) and 2 inbred strains (MON/Jms/Gbs Slc and MGS/Sea) were evaluated for their susceptibility, but they did not show significant differences in the number of worms recovered. However, Mongolian gerbils captured in Mongolia showed a lower number of worms recovered than HU strain. Lastly, the different source of protoscoleces were evaluated. The number of

worms were compared using protoscoleces from different sources of *E. multilocularis*. It was suggested that more passages by secondary hydatidosis may cause the change of cyst structure and thus resulting in a decrease of the viability of protoscoleces.

In chapter II, an anthelmintic (praziquantel) trial to adult *E. multilocularis* was performed using the alternative definitive host model. The model would be useful in in vivo evaluation for anthelmintics, replacing for trials using dogs.

In chapter III, the model was applied to *E. vogeli* using Mongolian gerbils and the development of adult *E. vogeli* in the small intestine was observed. The gerbils treated with PTBA were orally inoculated with protoscoleces of *E. vogeli*. The worms recovered at 7 days post-infection (DPI) showed band formation. The second proglottid and genital primordia were observed at 14 DPI. Spermatozoa in seminal receptacle and cleavage of ova in uterus were observed in the second proglottid at 21 DPI. The hook formation in oncospheres was observed at 28 DPI and the embryophore formation at 34 DPI. Eggs including morphologically mature oncospheres in the feces were first detected at 35 DPI.

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