tion when stimulated with the recombinant BLVtax protein. Furthermore, BALB/c and C57BL/6 mice, and sheep immunized with the recombinant BLVtax produced BLVtax-specific antibodies and showed proliferative responses against BLVtax.

Epitope mapping using 30 synthetic peptides of 20 mer covering the whole BLVtax polypeptide sequence was carried out. A lymphocyte proliferation assay revealed that, an epitope was located at positions of 111–130 and 131–150 for C57BL/6 and BALB/c mice respectively. In the case of sheep, there were different epitopes for the individual sheep. B cell epitope (position 51–70) and T cell epitope (position 181–200) were identified in one of the sheep which developed the strongest immune response against the whole BLVtax protein. In addition, the peptide at position 11–30 induced a non-specific proliferative response in both immunized and unimmunized sheep.

These results suggest that BLVtax is an immunogenic protein for mouse and sheep, and there are different epitopes for each experimental animal used in this study. The recombinant tax protein or peptides bearing B and/or T cell epitopes are good candidates for vaccine development against BLV infection.

Improvement of coproantigen detection methods for the diagnosis of the definitive hosts of *Echinococcus multilocularis*

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Improvement of coproantigen detection technique for diagnosis of *E. multilocularis* infection in definitive hosts were carried out. Initially, 5 different monoclonal antibodies (mAb) against *E. multilocularis* (EmA7, EmA8, EmA9, EmA10 and EmA11), were compared and their specificities were evaluated. Those mAb were tested for their cross-reactivities with somatic antigen extracts and coproantigens of *Taenia hydatigena, E. granulosus* and *E. multilocularis* in sandwich ELISA (sELISA). Out of 5 mAb, EmA8, EmA9 and EmA11 reacted strongly with *E. multilocularis* antigens, and used in an immunohistochemical study to observe the changes in antigen distributions during the development of *T. hydatigena* and *E. multilocularis*. Similar antigen distribution was observed in *T. hydatigena* as in *E. multilocularis* in all mAb. However, the strongest reactions were recognized in the reproductive organs in *T. hydatigena*, while, in the tegment and parenchyma in *E. multilocularis*.

To improve the specificity and sensitivity of the coproantigen detection method, avidin-biotinylated peroxidase complex (ABC) method was applied to EmA9 based sELISA. sELISA's using either Rabbit anti-*E. multilocularis* excretory-secretory antibody or EmA9 as capture and biotinylated EmA9 as primary antibody (rAb/EmA9 or EmA9/EmA9, respectively) were determined to be the most appropriate for the detection of *E. multilocularis* coproantigens. The comparison between the OD values of ABC applied method and conventional method clearly showed an increase in reactivity in ABC sELISA's and, especially in rAb/EmA9, sELISA kept non-

specific reaction at a negligible level. EmA9/EmA9 sELISA had an increased non-specific reactivity, but was improved by using lower antibody concentration.

Furthermore, a Dot-ELISA, in which a nitrocellulose membrane coated with capture antibody, biotinylated antibody as primary antibody and streptavidin biotinylated horseradish peroxidase (HRP) complex were used to visualize the presence of the antigens, was developed for 'on the spot' diagnostic method in field survey situation. This method using EmA9/EmA9 system gave a slightly lower sensitivity compared to sELISA using plates, and showed to be useful as a simple diagnostic method.

It is concluded that the improved sensitivity of the detection of *E. multilocularis* coproantigens was achieved by the application of ABC methods, which lead to the development of a simple diagnostic method based on a Dot-ELISA for use in the field.

Time course of antibody response against the infection with eggs of *Echinococcus multilocularis* in mice.

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Although *Echinococcus* have been recognized as one of the causative agents of most serious parasitic zoonosis, information on the intermediate hosts infected via ingestion of eggs is poorly available. This is attributed by the limitation of the availability of safe animal laboratory facilities able to handle those eggs. In this study, experimental infection was done using viable *E. multilocularis* eggs and the time course of antibody response against the infection with eggs was evaluated in susceptible mouse strain (AKR/N).

A total of 35 mice were orally inoculated with approximately 300 parasite eggs. The mice were necropsied at 1, 2, 4, 6, 9, 12 and 16 weeks post-infection (WPI). Histological investigation of the parasite development showed the formation of laminated layer at 4 WPI, formation of brood capsule with the massive increase of germinal cells at 9 WPI and formation of protoscoleces at 16 WPI.

The antibody responses of mice to three parasite antigens prepared from immature and mature metacestodes and purified protoscoleces were evaluated by ELISA and immunoblotting. In ELISA, the time courses of antibody response to three antigens were similar, in which the increase of OD value was initially detected at 4WPI and was more apparent at 9 WPI. The observation indicated that a part of antigen molecules expressed in protoscoleces were also expressed in immature metacestode even before the formaion of protoscoleces. Comparison of the band pattern in immunoblotting of the antigens showed several common bands in these antigens. This also indicates that immature metacestode had already expressed the antigen molecules expressed in protoscoleces. Immunoblot using sera collected at various WPI showed that many of bands appeared from 4 or 9 WPI. The result indicated that host antibody response is strongly stimulated by the prolifera-