# CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST *ECHINOCOCCUS MULTILOCULARIS* AND DIAGNOSIS OF THE DEFINITIVE HOST BY COPROANTIGEN DETECTION

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Eleven monoclonal antibodies (mAbs) used in the study were produced by immunization of mice with either *E. multilocularis* (Em) adult somatic antigen or oral inoculation of protoscoleces. Reactivities of these mAbs in ELISA using somatic and E/S antigen of either the Em protoscolex or adult and in immunohistochemistry using the parasites at various developmental stage were categorized into 3 types. Regardless of whether somatic or E/S antigen was used, Type 1 mAbs reacted to protoscolex antigen strongly, Type 2 reacted to both (protoscolex and adult) antigens and Type 3 reacted to adult antigen strongly.

Sandwich ELISA, using a rabbit polyclonal catching antibody raised against Em adult E/S antigen and each type of mAb as a primary antibody, was performed for the detection of Em antigen in host feces. The coproantigen was detected in the feces of an experimentally infected jird by sandwich ELISA using Type 2 and 3, but not Type 1 mAbs. Therefore, Type 2 and 3 mAbs could be incorporated in coproantigen detection for diagnosis of Em infection in definitive hosts. EmA9 and EmA11 (Type 3 mAbs) were selected for further study.

A positive linear correlation was observed between the number of worms (14 days postinfection [DPI]) incubated and the amount of antigen excreted in *in vitro* culture. Furthermore, incubation of worms collected at different DPIs revealed that the antigen excreted per dish increased with worm development. These results indicated that the sandwich ELISA could be used as a potential tool to monitor the actual parasite burden and development in an experimental infection. In an experimental infection in 4 foxes, noticeable elevation of the coproantigen level was first recognized around 4 to 6 DPI. After that, the coproantigen level continued to increase until 14 DPI and then rapidly decreased, indicating that worm expulsion might have occurred around 14 DPI.

To evaluate the specificity of the assay, feces of a dog experimentally infected with *Taenia hydatigena* were tested by the sandwich ELISA. Slight cross-reactivity with the *T. hydatigena* coproantigen was observed in EmA9, while EmA11 did not show any cross-reaction. Western blot analysis showed that the 61 kDa molecule of *T. hydatigena* somatic antigen was recognized by EmA9, but not by EmA11.

Em antigens detectable by this ELISA were heat-stable and the presence of carbohydrate moieties in the epitope was confirmed by periodate treatment of the antigens.