

# IMMUNOPEROXIDASE STAINING OF ALVEOLAR HYDATID CYST FROM AN EXPERIMENTALLY INFECTED GERBIL

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**Abstract.** *Echinococcus multilocularis*, the small fox tapeworm, has an extensive geographical range in the northern hemisphere where foxes and small rodents represent natural hosts. The larval stage of this parasite, alveolar echinococcosis (AE), is an emerging zoonosis of increasing importance. It is a serious human illness which is often misdiagnosed as hepatic cancer. If not identified at an early stage of parasite development it can lead to the death of patients. Histological examination of biopsies is one of the classical methods of diagnosis. In this study, in order to gain unequivocal histopathological diagnosis of AE, the immunoperoxidase staining technique was performed on routinely processed histological sections of an experimentally infected gerbil, using rabbit anti-*E.multilocularis* protoscolex IgG labelled with horseradish peroxidase. Demonstration of AE antigen was achieved by dark brown stain of cyst membranes against a blue background of the host liver cells stained with hematoxylin.

## INTRODUCTION

Alveolar echinococcosis (AE) caused by the larval stage of *Echinococcus multilocularis*, is a highly lethal zoonotic infection in northern climates. A great variety of animals, usually prey species of the definitive hosts, act as intermediate hosts, in which the embryo seems invariably to localize and develop in the liver (Raush, 1995). The cysts are gray-white, consisting of thin membranes; the delicate germinal layer is supported externally by the laminated layer. Demonstration of these membranes and their antigens can be useful in diagnostic pathology. In the present study, visualization of AE antigens was achieved by immunoperoxidase staining of liver paraffin sections.

## MATERIALS AND METHODS

Liver tissues of a gerbil, experimentally infected with alveolar hydatid cysts, were fixed in formal saline. After paraffin embedding, 4 micrometer sections were cut and placed on poly-l-lysine coated slides to dry at 37°C overnight. Then, the slides were dewaxed in xylene for 10 minutes, rehydrated in 100% ethanol, 70% ethanol for 2 minutes each, prior to rinsing in tap water.

In order to remove endogenous peroxidase, the slides were placed in 1% H<sub>2</sub>O<sub>2</sub> in methanol for 30 minutes. Later, they were washed in 1% egg albumin 2x15 minutes to reduce background staining. Then,

rabbit anti-*E.multilocularis* protoscolex IgG labelled with horseradish peroxidase (HRPO) were layered onto sections in 1:500 dilution in 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 90 minutes at room temperature (or 1:800 dilution overnight at 4°C).

After incubation, sections were rinsed in egg albumin for 15 minutes and then washed in Tris/HCl buffer 2x10 minutes. Afterwards, 3,3'-diaminobenzidine tetrahydrochloride (DBA) was layered onto sections for 3-10 minutes. After washing in tap water, sections were counterstained with hematoxylin 30-60 seconds, rinsed in tap water to blue up. Finally, sections were dehydrated through graded series of alcohol to xylene, and mounted in DPX.

## RESULTS

Strong staining was observed using rabbit anti-*E.multilocularis* protoscolex IgG labeled with horseradish peroxidase at 1:500 dilution. Cyst membranes stained dark brown against a blue background of the host liver cells stained with hematoxylin (Fig 1).

## DISCUSSION

Immunoperoxidase microscopy technique uses antibody conjugated to the enzyme peroxidase to detect antigen. The enzyme label is localized by reaction with an invisible product (Johnstone and Thorpe, 1982). This technique has been used for several parasitic infections, such as assessing *Dirofilaria immitis* in dogs (Tanaka and Atwell, 1991), immunologic diagnosis of

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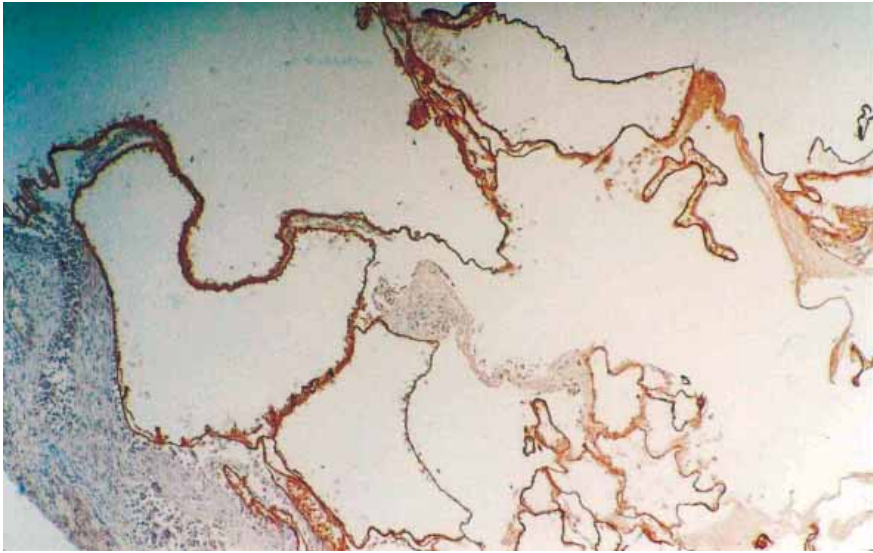


Fig1- Immunoperoxidase staining of *Echinococcus multilocularis* from the liver of an experimentally infected gerbil using specific antibody; dark brown stain of alveolar cyst membranes against blue background of liver cells.

*Schistosoma japonicum* (Zhou *et al*, 1988), detection of antibodies in cerebrospinal fluid in neurocysticercosis (de Andrade *et al*, 1996), identification of *Leishmania donovani* in canine tissues (Ferrer *et al*, 1988), and diagnosis of bovine abortion caused by *Neospora caninum* (Thurmond *et al*, 1999).

In this study, detection of AE was done by immunoperoxidase staining of routinely processed histological sections of liver from an experimentally infected gerbil. By immunoperoxidase staining, AE antigens can be demonstrated, even in an early cyst, before forming any protoscolexes.

The application of this technique for recognition of AE from hydatid cysts and its utility in diagnostic pathology, especially in geographical areas where both species occur, needs further investigation.

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