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A latex agglutination test for the detection of *Echinococcus multilocularis* coproantigen in the definitive hosts

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Abstract

A latex agglutination test for detecting *Echinococcus multilocularis* coproantigen in definitive hosts was developed using latex beads sensitized with EmA9 monoclonal antibody raised against somatic antigens of adult *E. multilocularis*. A primary test (LA 1) was performed on 82 fecal samples of necropsied foxes, of which 46 were infected, and resulted in 61% sensitivity and 86% specificity. To increase the sensitivity, 4 ng/mL of excretory/secretory antigens of adult worms was added to the samples in a secondary test (LA 2), resulting in 91% sensitivity and 61% specificity. The positive predictive value of the LA 1 test and the negative predictive value of the LA 2 test were both 85%. The combination of the LA 1 and LA 2 tests is applicable and practical for use in situations that require quick diagnosis or screening based on the following interpretation: the samples that are positive in the LA 1 test are positive; the samples that are negative in the LA 2 test are negative; and the samples that are negative in the LA 1 test and positive in the LA 2 test are classified as suspicious.

Keywords: *Echinococcus multilocularis*; Latex agglutination test; ELISA; Coproantigen; Diagnosis; Definitive host

1. Introduction

Echinococcus multilocularis, one of the most serious zoonotic parasites, is widely distributed in the northern hemisphere, including Hokkaido, Japan (Eckert et al., 2001). Humans are infected by ingesting eggs derived from the feces of definitive hosts. In Hokkaido, the prevalence of *E. multilocularis* in red foxes, the main definitive host, has been approximately 40% over the last two decades. Moreover, infections of domestic dogs, which are another potential infectious source for humans because of their close proximity, have been reported not only on Hokkaido, but also on the main island of Japan (Kamiya et al., 2007; Morishima et al., 2006; Nonaka et al., 2006). In response to the deteriorating situation, a reporting system for canine echinococcosis has been enforced in Japan since October 2004; as of March 2007, seven cases have been detected.

The ability to perform a rapid and on-site diagnosis/screening for infection in definitive hosts would be beneficial for small animal practitioners in their risk management; however, this is difficult to perform with the currently available diagnostic tools. Fecal egg examination is inaccurate for *Echinococcus* species because of the morphological similarity of eggs among taeniid species and the intermittent excretion of eggs even after maturity (Eckert and Deplazes, 2001;

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Nonaka et al., 1996; Yamashita et al., 1956). Coproantigen detection methods have been developed for the diagnosis of *Echinococcus* species using either monoclonal or polyclonal antibodies (Deplazes et al., 1999; Sakashita et al., 1995). Although a certain level of cross-reactivity with *Taenia* spp. infections has been recognized (Allan and Craig, 2006; Malgor et al., 1997), the validity of the assays was ascertained in *E. multilocularis* (Deplazes et al., 1999; Morishima et al., 1999), *Echinococcus granulosus* (Malgor et al., 1997), and *Echinococcus vogeli* infections (Matsuo et al., 2000). However, the assays were based on sandwich enzyme-linked immunosorbent assay (ELISA); thus, it takes one to several days to obtain results using a commercial kit or by outsourcing, respectively. Reliable DNA diagnostic methods are available; however, on-site diagnosis is difficult in most cases. We developed a latex agglutination test for detecting *E. multilocularis* coproantigen for on-site diagnosis.

2. Materials and methods

2.1. Parasitological examination and fecal sample collection

The samples used were obtained from 82 foxes shot in and around Sapporo (Ebetsu, Kita Hiroshima, Nanporo and Otaru) during 1997 to 1999 and were frozen at −80 °C for more than 10 days to sterilize infectious eggs of *E. multilocularis*. The intestinal tract of each fox was removed, and parasitological examination was performed on the intestinal contents and the scraping of the mucosa of the whole small intestine and colon under a stereomicroscope. The number of *E. multilocularis* found was counted. Fecal samples from the rectum were mixed with approximately equal volumes of 1% formalin, incubated at 70 °C for 12 h, and kept at room temperature. They were used for latex agglutination tests and sandwich ELISA.

2.2. Sensitization of latex particles

Polybead carboxylate microspheres were coupled with EmA9, a monoclonal antibody raised against adult *E. multilocularis* somatic antigen (Kohno et al., 1995), according to the manufacturer's instructions for the Carbodiimide Kit for Carboxylated Microparticles (Polysciences, Inc.). Briefly, 0.5 mL of a 2.5% carboxylated latex particle (diameter 1.0 μm) solution was washed twice with carbonate buffer by centrifugation at 13,000 × *g* for 6 min, and the supernatant was removed after each wash. The sediment was washed three times with phosphate buffer in the same manner. The sediment was resuspended with 0.6 mL of phosphate buffer and then stirred for 3.5 h on a wave shaker (MINI WAVE, Iuchi) with an equal volume of carbodiimide solution. The mixture was washed three times with borate buffer. After the supernatant was removed, it was resuspended with 1.2 mL of phosphate buffer that contained 60 μg of EmA9 and stirred overnight at room temperature. It was then centrifuged, and the supernatant was removed. One milliliter of 0.1 M ethanolamine was added to the sediment and stirred for 30 min. The mixture was washed, and the sediment was stirred with 1 mL of BSA solution for 30 min in the same manner. After the supernatant was removed, it was resuspended with 1 mL of storage buffer (final concentration: 1.75%) and stored at 4 °C. The latex particles sensitized with EmA9 were used within 2 weeks.

2.3. Latex agglutination tests

Latex agglutination test 1 (LA 1): samples were diluted to 0.13 g/mL with 0.1% Tween 20 in PBS and centrifuged. The supernatant (15 μL) and sensitized latex particles (5 μL) were mixed on a glass slide. Agglutination was checked after 5 min, and the results were classified into three categories by degree of agglutination: −, no agglutination; +, small agglutination masses formed; ++, large agglutination masses formed. Samples that were + or ++ were considered positive for agglutination (Fig. 1).

Latex agglutination test 2 (LA 2): to increase the sensitivity of LA 1, fecal samples were diluted with buffer containing 4 ng/mL of excretory/secretory antigen of adult *E. multilocularis* (EmES antigen) (Sakashita et al., 1995), and a latex agglutination test similar to LA 1 was performed. However, agglutination was checked after 7 min.

2.4. Sandwich ELISA

To compare the results of LA 1 and LA 2 with those of a standard technique, a sandwich ELISA was performed for coproantigen detection using the method of Morishima et al. (1999). A cut-off value to discriminate between positive and negative samples was calculated as 0.111, which was the mean optical density (OD) plus three standard deviations of fecal samples from silver foxes uninfected with *E. multilocularis* (Kaji mink, Fukagawa).