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Field evaluation of an intravital diagnostic test of *Echinococcus multilocularis* infection in red foxes

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Abstract

Echinococcus multilocularis parasitizes the small intestine of red foxes *(Vulpes vulpes)* and other carnivores, and has a wide distribution throughout the northern hemisphere. This cestode is the causative agent of human alveolar echinococcosis, a life-threatening helminth zoonosis. In 2000–2002, 2130 red foxes were examined for its presence in Slovakia, with a total prevalence of 30.7%. The data on occurrence were obtained by the combination of necropsy of small intestines from red foxes and coproantigen detection in faecal samples. The correlation between the number of detected specimens and the value of optical density of copro-ELISA test was found. When worm burdens were low (1–25 specimens) the sensitivity of the method was $31.3 \pm 8.64\%$, when worm burdens were >50 specimens, $81.8 \pm 0.66\%$, and with high worm burdens (>1000 specimens) the sensitivity reached $100 \pm 0.34\%$. *E. multilocularis* presence was detected using the nested PCR method from the eggs in the faecal samples with a 100% specificity. In epidemiological surveys of this zoonosis, it is of crucial importance to detect animals with a high level of infection, which are responsible for the bulk of environmental contamination. The advantage of copro-ELISA test lies in allowing the intravital diagnostics to be employed within the epidemiological survey of *E. multilocularis* occurrence in the protected and urban areas.

Keywords: *Echinococcus multilocularis*; Cestoda; Red fox; Diagnosis; Coproantigen ELISA; Sedimentation and counting technique; Nested PCR

1. Introduction

Larval stages of *Echinococcus multilocularis* may cause human alveolar echinococcosis; a rare disease, which is lethal in untreated patients. Over a considerably short time period, the cestode has spread throughout the territory of the Slovakia in red foxes, with the prevalence increased from 24.8% in 2000 to 32.8% in 2002 (Dubinský et al., 2001; Miterpáková et al., 2003).

At present, several methods for diagnosis of echinococcosis in definitive hosts are employed.

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The flotation method enables the detection of only the presence of *Taenia*-type eggs, as they are further morphologically undistinguishable. Parasitological examination of the small intestine at necropsy is considered to be the most reliable method; however, it requires very strict safety rules and is labour-consuming. The method includes the intestinal scraping technique (IST); the sensitivity of which reaches 78% and specificity almost 100%. Another method, the sedimentation and counting technique (SCT), is due to its high sensitivity and specificity, being regarded as ''gold standard'', and is used for quantitative determination of *E. multilocularis* in the host intestine (Eckert, 2003). The above methods (SCT and IST) are suitable only for the *post mortem* diagnostics.

New approaches have been implemented for intravital diagnostics. The coproantigen detection from faecal samples of the definitive hosts using the ELISA method (copro-ELISA) is regarded as an alternative method with a sensitivity ranging from 84% to 95.5% and specificity over 96% (Eckert, 2003). The coproantigen ELISA can detect *E. multilocularis* antigens in faecal material already during the prepatent period and coproantigen excretion is closely correlated with the presence of intestinal immature and mature parasitic stages and their numbers (Eckert and Deplazes, 2001). The DNA detection of *E. multilocularis* in faeces is highly sensitive (89–94%), with its specificity almost 100% (Eckert, 2003). According to Dinkel et al. (1998), the strong point of the method is the detection of DNA isolated from eggs or the whole tapeworm segments. Nested PCR method enables identification of the causative agent in case of the positive coproantigen findings of *Taenia*-type eggs in faeces. Detection of circulating antibodies is not suitable for estimating the actual parasite prevalence in populations of foxes or other definitive hosts because of insufficient correlation between the prevalence of serum antibodies and the presence of intestinal worm burdens (Eckert and Deplazes, 2001).

The aim of the present study was to assess the validity of coproantigen ELISA to diagnose *E. multilocularis* infection in red foxes in the field conditions of Slovakia and to compare this method with the sedimentation and counting techniques with respect to the intensity of infection.

2. Material and methods

2.1. Animals and sampling

A total of 2130 samples of faeces or intestine from red foxes (*Vulpes vulpes*) negative for rabbies from different parts of the Slovak Republic were collected in spring and autumn in the period of 2000–2002. The material prior to the examination was frozen at −80 °C for 7 days to reduce risk of infection (Veit et al., 1995).

2.2. Parasitological examinations

Two techniques were used for detecting of *E. multilocularis* in red foxes and for determination of prevalence and intensity of infection.

2.2.1. The sedimentation and counting technique (SCT)

Small intestine was cut into five pieces and incised longitudinally. The mucosa layer was stripped into water. After several washings on a 1.5 mm mesh size sieve, the sediment had been examined in small portions in Petri dish under a stereomicroscope (Raoul et al., 2001). Worm burdens were classified as low (1–25; 25–50; 51–100 specimens), medium (101–1000 specimens) and high worm burdens (>1000 specimens) according to Ewald and Eckert (1993). Worm specimens were identified as *E. multilocularis* following morphological criteria of Vogel (1957) and Thompson (1995). A total of 1328 red foxes were examined by this technique.

2.2.2. The intestinal scraping technique (IST)

The small intestine was opened in full length. After removal of rough content 15 deep mucosal scrapings were taken from the proximal, middle and posterior parts of the small intestine and examined microscopically (Deplazes and Eckert, 1996). A total of 599 red foxes were examined by this technique.

2.3. Coproantigen detection

Coproantigens of *E. multilocularis* were detected by sandwich enzyme-linked immunosorbent assay (copro-ELISA) using Chekit®Echinotest (Dr. Bommeli AG, Liebefeld-Bern, Switzerland). The preparation of 745