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VERSITA

Occurrence of *Echinococcus multilocularis* in red foxes from the Carpathian regions of Slovakia and Poland

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Abstract

The extensive distribution of *Echinococcus multilocularis* cestode from endemic alpine areas to the parts of Central Europe has been recorded in recent years. The first confirmed finding of *E. multilocularis* in Slovakia was recorded in 1999 in the area adjacent to the Polish border. At present, this serious zoonosis occurs almost across the whole territory of Slovakia. The occurrence of these tapeworms in red foxes (*Vulpes vulpes*) at the border regions of Slovakia and Poland has been monitored. In these districts, out of 152 faecal samples examined, 36.2% were positive for the coproantigen-ELISA. With the sedimentation and counting technique the prevalence of *E. multilocularis* in red foxes was up to 38.8%. The examination of foxes from neighbouring districts revealed worm burden ranging from 1–15,000 specimens, but the majority of animals harboured medium number of tapeworms. In the Small Carpathian and Sub-Carpathian regions of Poland, out of 65 samples examined, 13.8% were coproantigen positive. Using the small intestine scraping method only 6.1% prevalence of *E. multilocularis* in red foxes was determined, mostly with a high worm burdens over 1,000 specimens. The results suggest possible transborder transmission of *E. multilocularis*, the causative agent of serious alveolar echinococcosis.

Key words

Echinococcus multilocularis, red foxes, zoonosis, coproantigen-ELISA

Introduction

The expansive spread of *Echinococcus multilocularis* from endemic alpine areas to the parts of the territory of France, most of Germany, Switzerland and Austria and latterly to neighbouring territory of the Czech Republic and Poland, according to assumption, has not been stopped (Eckert 1997). The first confirmed finding of *E. multilocularis* in Slovakia was in 1999 at the border area with Poland (Dubinský *et al*. 1999). Now this serious zoonosis occurs throughout most of the territory of Slovakia (Dubinský *et al*. 2001). Recent studies have shown that *E. multilocularis* has a wider geographic range than previously anticipated. There is evidence for growing populations of red foxes in some areas, for increasing invasion of cities by foxes and also for establishment of the parasite cycle in urban areas (Gloor *et al*. 2001). These and other factors may lead to an increased infection risk for humans. Significant progress has been made in the development of sensitive and specific new techniques for the *intra vitam* and *post mortem* diagnosis of intestinal *E. multilocularis* infection in definitive hosts. Coproantigens are detectable during prepatent and patent periods in definitive hosts, and disappear within a few days after elimination of the cestodes from the host. Despite the tendency of higher ELISA absorbance values in heavily infected hosts, quantification of the infection intensity is not possible (Deplazes and Eckert 2001).

The objective of this work was to investigate the occurrence of *E. multilocularis* in red foxes at the border regions of Slovakia and Poland.

Materials and methods

During the period of 2000 to 2002 a total of 217 red foxes (*Vulpes vulpes*) from the border territory of Slovakia and Poland were examined.

From the Slovak districts bordering Poland (Poprad, Kežmarok, Stará Ľubovňa, Bardejov, Svidník, Stropkov, Medzilaborce and Snina) 152 red foxes were examined. Following capture, the red foxes were transported to the State Veterinary Institute and examined for the presence of rabies virus. The small intestine and faecal samples were collected and

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frozen at –80°C for at least 7 days to minimise infection risk (Veit *et al*. 1995). The intestines and faeces of foxes negative for rabies were investigated using the following methods:

(1) Sedimentation and counting technique (SCT) described by Hofer *et al*. (2000). According to the method of Ewald and Eckert (1993), worm burdens were classified as low (1–100 worms), medium (101–1,000 worms) and high (>1,000 worms).

(2) Detection of *Echinococcus* coproantigen by enzyme-linked immunosorbent assay. A commercially available test kit was used (CHEKIT® – Echinotest, Dr Bommeli AG, Switzerland). The preparation of faecal samples and estimation of the coproantigen level was carried out according to the manufacturer's instructions.

From the Carpathian region of Poland bordering Slovakia 65 red foxes were examined from 2 provinces (Small Carpathian and Sub-Carpathian) and 9 administrative districts (Sanok, Dębica, Jasło, Krosno, Brzozów, Gorlice, Rzeszów, Limanowa and Bieszczadzki).

The intestines and faeces of animals were isolated and examined for the presence of *E. multilocularis* using:

(1) Intestinal scraping technique (IST) according to Deplazes and Eckert (1996). Fifteen deep mucosal scrapings were taken from the proximal, middle and posterior parts of the small intestines and examined microscopically. Worm specimens were identified as *E. multilocularis* using the morphological criteria of Vogel (1957) and Thompson (1995).

(2) Detection of *Echinococcus* coproantigens by "double sandwich" ELISA according to Machnicka *et al*. (2003) (different technique as used before). Briefly: Flat-bottom microtitrate plates (Nunc, MaxiSorp) were coated overnight at 4°C with somatic antigens of *E. multilocularis* containing 2 µg protein/ml coating buffer. After washing, the rabbit immunoglobulins anti-*E. multilocularis* containing 20 µg protein/ml diluted in PBSTA (PBST + 0.5% bovine serum albumin) was added and incubated at 37°C for 1 h. Next, the faecal samples diluted 1:10 in PBST (PBS + 0.05% Tween-20) were added and incubated at 37°C for 1 h. After washing, rabbit anti-*E. multilocularis* immunoglobulins conjugated to HRP (horseradish peroxidase), diluted 1:100 in PBSTA were added. After 1 h incubation at 37°C and washing, the substrate OPD (orthophenylenediamine) was added. The plates were read using automatic 96-well reader (Organon Teknika) at 492 nm.

The results were evaluated statistically by the chi-square test. Comparison among two techniques was done using Fisher's Exact test. The differences among the groups were considered significant at the values of $p<0.05$.

Results

Out of 152 faecal samples examined by coproantigen-ELISA detection, 36.2% were positive. Using the sedimentation technique the prevalence of *E. multilocularis* in red foxes was 38.8% (Table I). The examination of the bordering locality in Slovakia revealed mostly medium worm burdens with a wide range (1 to 15,000 parasites). Differences in prevalence between regions were statistically not significant ($p = 0.6966$, $df = 7$, $\chi^2 = 4.7$). Also no significant differences were found between the results of the SCT and coproantigen-ELISA, using Fisher's Exact test ($p = 0.82$).

In the Small Carpathian and Sub-Carpathian regions of Poland, using immunoenzyme coproantigen detection, out of 65 samples examined 13.8% were positive. With the small intestine scraping method only 6.2% prevalence of *E. multilocularis* in red foxes was determined, mostly with a high worm burden (over 1,000 specimens) (Table II). Differences between examined regions were statistically not significant ($p = 0.61$, $df = 8$, $\chi^2 = 6.33$). Non-significant differences were found between IST and coproantigen-ELISA methods according to Fisher's Exact test ($p = 0.23$).

Topography of the occurrence of echinococcosis in red foxes in the Carpathian region of the Slovakia and Poland are marked on Figure 1.

By the coproscopical investigation *Mesocestoides* spp., suborder Ascaridata (*Toxocara* spp., *Toxascaris* spp.), *Trichuris* spp. and *Strongyloides* spp. were found. The *Taenia*-type eggs were detected in 16 faecal samples only.

Table I. Occurrence of *Echinococcus multilocularis* in red foxes from Slovak districts bordering Poland

Districts in Slovakia	Foxes examined	SCT*		Coproantigen-ELISA**	
		positive	%	positive	%
Bardejov	29	13	44.8	12	41.3
Kežmarok	19	11	57.9	11	57.9
Medzilaborce	5	1	20.0	0	0
Poprad	27	7	25.9	7	25.9
Snina	16	8	50.0	8	50.0
Stará Ľubovňa	13	2	15.4	0	0
Stropkov	13	6	46.2	6	46.2
Svidník	30	11	36.7	11	36.7
Total	152	59	38.8	55	36.2

*SCT – sedimentation and counting technique, **coproantigen-ELISA – immunoenzyme coproantigen detection.

Table II. Occurrence of *Echinococcus multilocularis* in red foxes in Small Carpathian and Sub-Carpathian regions of Poland

Districts in Poland	Foxes examined	IST*		Coproantigen-ELISA**	
		positive	%	positive	%
Sanok	14	1	7.14	4	28.6
Jasło	7	0	0	1	14.3
Dębica	8	1	12.5	1	12.5
Krosno	7	0	0	1	14.3
Bieszczadzki	8	2	25.0	2	25.0
Brzozów	6	0	0	0	0
Gorlice	7	0	0	0	0
Rzeszów	5	0	0	0	0
Limanowa	3	0	0	0	0
Total	65	4	6.2	9	13.8

*IST – intestinal scraping technique, **coproantigen-ELISA – "double sandwich" immunoenzyme coproantigen detection.

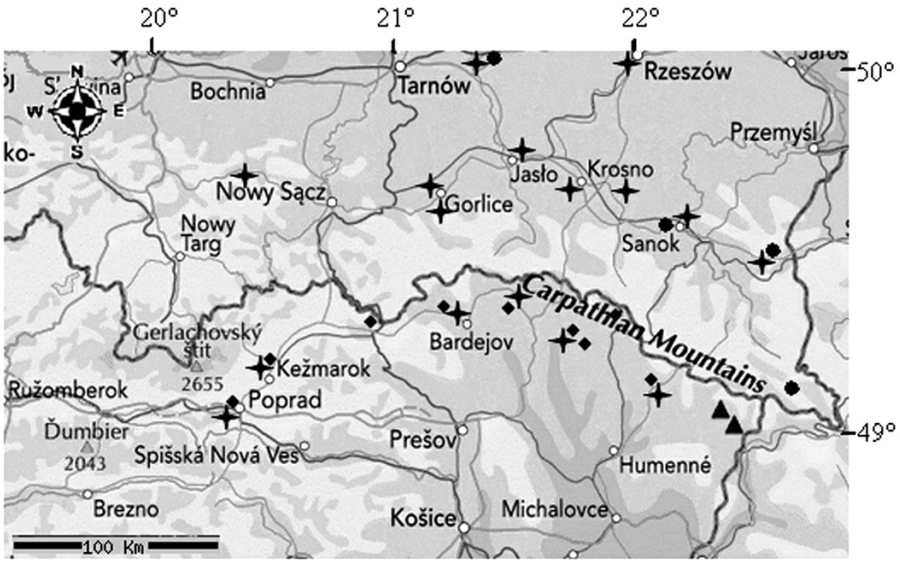

Fig. 1. Occurrence of *Echinococcus multilocularis* in Slovakia and Poland: first PCR positive findings in 1998 (▲), scraping technique in 2002 (●), sedimentation method in 2000–2002 (♦), coproantigen findings by ELISA in 2000–2002(✦)

Discussion

The spread of *Echinococcus multilocularis* in red foxes in Central Europe is an example of transborder transmission of helminthozoonoses. The borders between the Slovak Republic and its neighbouring countries are formed by the Danube and the Morava rivers in the south-west (Austria) and the Carpathian Mountains in the west and north (Czech Republic and Poland); 32.3% of the total Slovak border is shared with Poland. As much of the landscape consists of forest and agricultural areas, it is evident that the conditions for transborder transmission of *E. multilocularis* from neighbouring countries to the Slovak Republic are favourable in addition to the High Tatra Mountains as a main barrier. Recent studies have reported a high prevalence of *E. multilocularis* in red foxes in the Czech Republic and Poland. In 1995 *E. multilocularis* was first reported in red foxes in the south-west of the Czech Republic (Kolářová *et al*. 1996) and a year before also in Poland (Malczewski *et al*. 1995). In subsequent years a rapid spread of *E. multilocularis* in red foxes in other regions of both countries was evident (Pavlásek *et al*. 1997, Malczewski *et al*. 1999).

Transborder transmission of *E. multilocularis* to Slovak territory began probably before 1998. *E. multilocularis* worms were first observed in faecal samples collected from red foxes in some regions in 1998/1999. In the Bratislava region only

one fox was positive at necropsy, while a few positive foxes were found in the northern districts on the Polish border. Coproantigen positive samples were also found in the Košice region in south-east Slovakia (Dubinský *et al*. 1999), while in 1999 specific DNA sequence of *E. multilocularis* was detected by nested PCR in two faecal samples from wolves in the same region (Martínek *et al*. 2000).

Our joint results refer to the probable transborder transmission of *E. multilocularis*. Similar ecological conditions on both sides of the Slovak-Polish border promoted the dissemination of this serious zoonosis. Growing populations of red foxes and their increasing immigration into urban areas are new risk factors for disseminating this parasite (Eckert and Deplazes 1999). The first three cases of autochthonous human alveolar echinococcosis were recorded in Slovakia recently (Kinčeková *et al*. 2001, 2002, 2005).

The results of the present study demonstrate that the disease is spreading, emphasising the need for appropriate control strategies. From this aspect the sensitive and specific appropriate diagnostic techniques for the detection of *E. multilocularis* in definitive hosts is important for the study and control of echinococcosis.

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