Journal of Helminthology (2006) **80,** 243–247 DOI: 10.1079/JOH2006354

Echinococcus multilocularis in the red fox *Vulpes vulpes* from the East Carpathian region of Poland and the Slovak Republic

P. Dubinský[1]*, A. Malczewski[2], M. Miterpáková[1], J. Gawor[2] and K. Reiterová[1]

[1]Parasitological Institute of the Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovak Republic: [2]W. Stefański Institute of Parasitology of the Polish Academy of Sciences, Twarda 51/55, 00818 Warsaw, Poland

Abstract

The occurrence of *Echinococcus multilocularis* in the Poland–Slovak frontier zone of the East Carpathian region was assessed, for comparison with that in adjacent regions in both countries. A total of 392 red foxes from Poland and 427 red foxes from the Slovak Republic were examined from 2001 to 2004. Significant differences in prevalences were observed in foxes captured from the borderland and adjacent zones in both countries. The mean prevalence of *E. multilocularis* in the Polish borderland reached 45.7 ± 18.6% and in the Slovak border 35.0 ± 10.7%. In both countries, the prevalence of *E. multilocularis* in red foxes from adjacent districts, outside the frontier Carpathian region, was considerably lower (18.9 ± 9.2% in Poland and 20.8 ± 9.0% in Slovakia). These differences are probably due to geomorphological and ecological factors, which contribute to the survival of the tapeworm eggs and the subsequent spread of infection. The Carpathian regions of northeast Slovakia and southeast Poland are characterized by specific climatic conditions such as low mean annual air temperatures, low temperatures in active soil surfaces, high soil humidities and a high mean annual rainfall.

Introduction

Alveolar echinococcosis has been considered for almost a decade as one of the most serious diseases. According to the Directive No. 2003/99 ES of the European Parliament and the EU Council on monitoring of zoonoses and their causative agents, it was classified into category 'A', requiring permanent monitoring of its occurrence in all EU member states. A causative agent of alveolar echinococcosis, the tapeworm *Echinococcus multilocularis*, has been intensively researched in Europe with an increasing number of human infections recorded in the early 1990 s.

Echinococcus multilocularis in Central Europe was detected for the first time in the red fox, in the north of Poland in 1995 (Malczewski *et al.*, 1995). Subsequently, further epidemiological studies were undertaken in Poland, where in 1993–1998, a total of 2951 red foxes were examined in 43 of 49 administrative units (voivodships). Infected foxes were found in 18 voivodships with a mean prevalence of 2.6%. The prevalences varied significantly between regions (Malczewski *et al.*, 1999).

Until 1999, no systematic study had been undertaken on the occurrence of *E. multilocularis* in Slovakia. Several authors who conducted studies on the parasitic fauna of wild carnivores and rodents did not succeed in finding any mature or larval stages of *E. multilocularis* (Baruš, 1961; Mituch, 1963, 1964, 1972; Prokopič, 1965). Only after the parasite was found in neighbouring Poland and the Czech Republic, was a targeted examination of red foxes in Slovakia started. The first record of *E. multilocularis* in the red fox in Slovakia was in 1999, in the Senec, Gelnica

*Fax: +421 55 63 31414
E-mail:dubinsky@saske.sk

and Košice districts in the south and in the bordering districts of north-eastern Slovakia (Dubinský *et al.*, 1999). An epidemiological survey of *E. multilocularis* in Slovakia in 2000–2002 (Miterpaková *et al.*, 2003), showed that the highest prevalence was recorded in northern districts of Slovakia bordering Poland.

The aim of the present study was to compare the occurrences of *E. multilocularis* in red foxes in border areas of the East Carpathian region and adjacent areas of Poland and the Slovak Republic.

Materials and methods

The study sites were divided into two zones (fig. 1). Zone I (borderland) includes the contiguous border districts of Poland (Jaslo, Krosno, Sanok, Lesko and Bieszczady) and of the Slovak Republic (Svidník, Stropkov, Medzilaborce, Humenné and Snina), whose territories are up to 50 km from the state border. The total area of Polish districts in Zone I represents 4953 km^2 and of Slovak districts 2925 km^2. Geologically, this border zone belongs to the East Carpathian mountain range. The Polish and Slovak regions are directly bounded by the Outer East Carpathian, represented by Laborecká Vrchovina upland and Bukovské Vrchy mountains on the Slovak side and Beskyd Niski and Bieszczady on the Polish side. The border Carpathian region is characterized by low mean annual air temperatures (4–6°C), low mean soil surface temperatures (3–5°C) and high annual precipitations (90–1300 mm). The landscape is mainly mountainous, including mixed and coniferous forests and pastures. The altitude ranges between 600 and 1350 m above sea level.

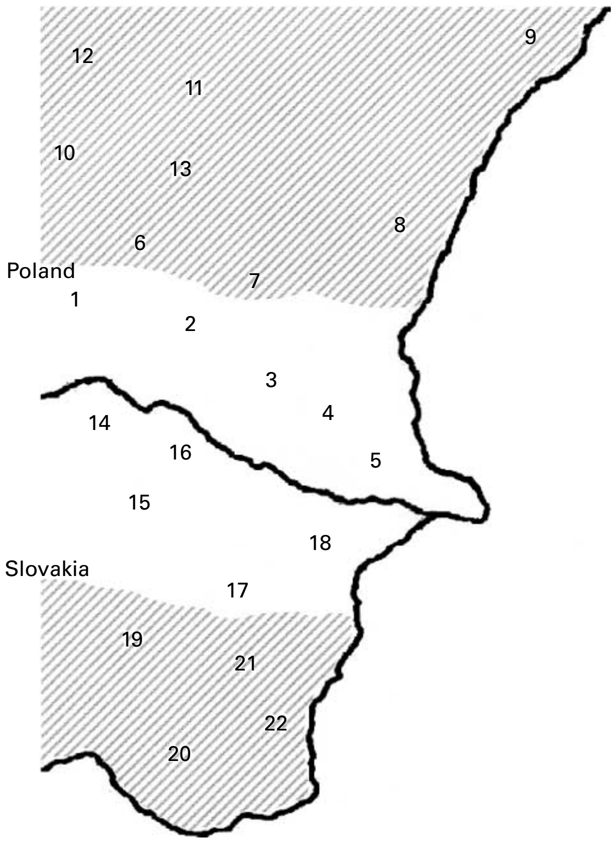

Fig. 1. Map of Poland-Slovak borderline to show sampling sites of red foxes in Zones I (borderland) and II (adjacent areas) of Poland and Slovakia during 2001–2003/4. (□, Zone I; ▨, Zone II; 1, Jaslo; 2, Krosno; 3, Sanok; 4, Lesko; 5, Biesczady; 6, Stryzov; 7, Brzozow; 8, Przemysl; 9, Lubaczow; 10, Debica; 11, Kolbuszow; 12, Mielec; 13, Ropczyce; 14, Svidník; 15, Stropkov; 16, Medzilaborce; 17, Humenné; 18, Snina; 19, Vranov nad Topl'ou; 20, Trebišov; 21, Michalovce; 22, Sobrance.

Zone II (adjacent area) includes eight Polish districts situated to the north of Polish Zone I (Strzyżów, Brzozów, Przemyśl, Lubaczów, Dębica, Kolbuszów, Mielec and Ropczyce) and four Slovak districts situated to the south of Slovak Zone I (Vranov n. Toplou, Trebišov, Michalovce and Sobrance). The territory of both areas is situated 50 to 140 km from the common state border. The area of the districts of the Polish Zone II covers 6544 km^2, and of Slovak districts 3400 km^2. Climatic conditions in adjacent zones are higher mean annual air temperatures (7–9°C in the Polish zone, 8–10°C in the Slovak zone) and lower mean annual rainfall (450–650 mm). Adjacent regions of both countries are predominantly agricultural with a high proportion of arable land and altitude from 200 to 560 m in the Polish area and from 94 to 800 m in the Slovak area.

A total of 392 red foxes were examined in Poland (265 in Zone I, 127 in Zone II) and 427 in the Slovak area (220 in Zone I, 207 in Zone II). In the Polish area, 5.4 red foxes in Zone I and 1.9 in Zone II were examined per 100 km^2, compared with 7.5 red foxes in Zone I and 6.1 in Zone II per 100 km^2 in Slovakia.

Of 427 red foxes collected from Slovakia, 358 were examined by necropsy and 69 by coproantigen detection. In Poland, necropsies were performed on all 392 foxes examined, using modified sedimentation and counting techniques (SCT) as previously described by Raoul *et al.* (2001).

The small intestine of each fox was cut into five sections and incised longitudinally. Each section was placed in a 1 l bottle containing water and the mucosa layer was stripped off manually. After shaking and 30 min of sedimentation, the supernatant was removed and the sediment was washed with water on a 1.5 mm mesh size filter to discard bigger particles. The filtered fraction was collected in a conical glass flask and sedimented for 1 h. The supernatant was removed and, after stirring, the sediment was examined in small portions of 10 ml in Petri dish under a stereomicroscope. Positive foxes were divided into four categories according to the number of parasites recovered: (i) low worm burden (1–10); (ii) medium worm burden (11–100); (iii) high worm burden (101–1000); (iv) and very high worm burden (>1000).

Coproantigens of *E. multilocularis* were detected in the faeces of red foxes, in cases where the small intestine was not available, using a commercial ELISA kit (Checkit®–Echinotest, Dr Bommeli AG, Switzerland). The preparation of faecal samples and estimations of coproantigen levels were carried out according to the manufacturer's instructions.

Two fundamental epidemiological characteristics, i.e. prevalence and mean worm burden per infected host

https://doi.org/10.1079/JOH2006354 Published online by Cambridge University Press

were evaluated in the field sites surveyed. These data were compared in foxes from the frontier zones of Poland and Slovakia along with the data obtained from the adjacent zones of both countries, with host sex being one of the factors considered. A non-parametrical Chi-square test was used to determine the statistical significance of the differences observed (StatSoft, Inc., 2001).

Results

During 2001–2004, a total of 392 red foxes in Poland and 427 in Slovakia were examined. The overall prevalence of *Echinococcus multilocularis* in Poland was higher than that in Slovakia (table 1). In both countries the prevalence in Zone I (borderland) was higher than in Zone II. Statistically significant differences were recorded between the prevalences only in the Polish Zone I and II ($\chi^2 = 10.90$, $P = 0.0009$), however they were not recorded in Slovakia. The prevalence in Zone I in Poland was higher than in Slovakia, although these differences did not differ significantly ($\chi^2 = 1.4129$, $P = 0.2346$). The number of infected red foxes per 100 km^2 in Poland (2.6) was almost the same as in Slovakia (2.4). The prevalences in Zone II (adjacent zone) in both countries did not significantly differ ($\chi^2 = 0.9602$, $P = 0.7567$), however in Poland, a smaller number of infected red foxes per 100 km^2 (0.4) was recorded than in Slovakia (1.3).

When comparing the prevalences of *E. multilocularis* in the bordering Zone I of both countries, despite annual fluctuations, significant differences were found (table 2). A decrease in prevalence in Poland was observed in 2002, while in Slovakia it was observed as late as 2003/04. Annual fluctuations in prevalences in Zone II of both countries were very similar (table 2). A significant decrease in prevalence was recorded in 2002 and this decrease also continued in Slovakia in 2003/4.

An evaluation of *E. multilocularis* prevalence depending on host sex (table 3) showed that in Poland the prevalence was higher in male (62.5%) than in female foxes (42.3%), while in Slovakia the infection rate in both genders was approximately at the same level (in males 28.0%, in females 30.3%).

In red foxes examined by the SCT method, worm burdens were also monitored (table 4). The mean burden of *E. multilocularis* in the surveyed Polish regions was 332.0 in Zone I and 300.0 in Zone II. In the Slovak territory, a total of 878 tapeworms were recovered, the worm burden being considerably higher in Zone I (1026 specimens) than in the adjacent Zone II (659 specimens).

Dividing infected foxes into four categories according to the number of tapeworms present showed that in Poland the highest number of foxes belonged to the group with low and medium worm burdens (36.2% and 36.2%, respectively). In Slovakia, the majority of foxes in both surveyed zones fell into the category of medium (35.5%) and high worm burdens (27.9%) (table 4).

Table 1. The prevalence of *Echinococcus multilocularis* in red foxes from individual districts of Zone I (borderland) and Zone II (adjacent area) in Poland and Slovakia from 2001 to 2004.

	Number of foxes		
	Examined	Infected	Prevalence (%)
Poland			
Zone I			
Jaslo	15	1	6.7
Krosno	71	35	49.3
Sanok	75	42	56.0
Lesko	34	19	55.9
Bieszczady	70	24	34.3
Total ± SD	265	121	45.7 ± 18.6
Zone II			
Strzyzów	43	11	25.6
Brzozów	14	3	21.4
Przemysl	43	5	11.6
Lubaczów	19	4	21.1
Debica	5	0	0
Kolbuszow	1	0	–
Mielec	1	0	–
Ropczyce	1	1	–
Total ± SD	127	24	18.9 ± 9.2
Overall total ± SD	392	145	37.0 ± 19.1
Slovakia			
Zone I			
Svidník	44	24	54.6
Stropkov	21	9	42.9
Medzilaborce	7	2	28.6
Humenné	64	21	32.8
Snina	84	21	25.0
Total ± SD	220	77	35.0 ± 10.7
Zone II			
Vranov n. Topl'ou	81	27	33.3
Trebišov	74	10	13.5
Michalovce	39	4	10.3
Sobrance	13	2	15.4
Total ± SD	207	43	20.8 ± 9.0
Overall total ± SD	427	120	28.1 ± 13.6

Discussion

An extensive epidemiological survey of *E. multilocularis* prevalence in red foxes in the territory of Poland since 1994 and in the Slovak territory since 2000 has demonstrated noticeable regional differences in the occurrence of this parasite. In the west of Poland a prevalence value of 1.2% was recorded while in the north-east, central and south of the country the prevalence reached 4.4% (Malczewski *et al.*, 1999). In Slovakia, the highest *E. multilocularis* prevalence was in the north of the country, in the Žilina region (57.6%) and in the Prešov region (42.2%) (Miterpáková *et al.*, 2003).

Table 2. The prevalence (%) of *Echinococcus multilocularis* in red foxes in Zones I (borderland) and II (adjacent areas) of Poland and Slovakia during 2001–2003/4.

	Prevalence (%)		
Zones	2001	2002	2003/4
Poland I	63.5	41.1	41.6
Slovakia I	47.2	48.7	28.6
Poland II	44.4	15.8	18.0
Slovakia II	38.2	22.0	10.8

https://doi.org/10.1079/JOH2006354 Published online by Cambridge University Press

Table 3. The prevalence of *Echinococcus multilocularis* in male and female red foxes in Poland and Slovakia from 2001 to 2004.

	Number of foxes		
	Examined	Infected	Prevalence (%)
Poland			
Male foxes	32	20	62.5
Female foxes	26	11	42.3
Slovakia			
Male foxes	175	46	28.0
Female foxes	185	56	30.3

The occurrence of human alveolar echinococcosis (AE) has been registered in Poland since 1992 and until 2004, 31 cases were reported. In Slovakia, four cases of AE have been reported to date (Kinčeková *et al.*, 2001, 2002, 2005).

In the 1960 s and 1970 s a long term study of the parasitic fauna of carnivores and rodents was concluded (Prokopič, 1965; Mituch, 1972; Mituch *et al.*, 1992) and *Echinoccocus multilocularis* was not recorded. However, it is more likely that throughout the past decades there has been a low or sporadic occurrence of *E. multilocularis* in the territory of both countries and the parasite was never diagnosed. A high increase in the prevalence of *E. multilocularis* in Slovakia resulted from an increase in the fox population after 2000, when oral vaccination of foxes against rabies commenced in the whole territory. Available data indicate that in 1993 the population of red foxes in Slovakia was 6154, which doubled to 13,331 in 1996 and increased again to 18,401 in 2002 (Anon., 1994, 1997, 2003).

In Poland, the population of red foxes has risen from 67,000 in 1995 to 164,000 in 2002 and 174,318 to March 2004. The increase in the number of foxes is likely to be related to the distribution of oral antirabies vaccines, which started in 1993 in western Poland bordering Germany and from 2002 in the whole territory.

In the current situation in the Carpathian region of Poland bordering the endemic area on the northeast Slovakia, the prevalence of *E. multilocularis* shows a similar trend on both sides of the border. In the Polish border regions, directly neighbouring Slovakia (Zone I), *E. multilocularis* prevalence in foxes reached a maximum value in 2001 and then sharply declined in 2002. In the Slovak regions of frontier Zone I, the highest prevalence was recorded in 2002 followed by a sharp decline in 2003/2004. Mean prevalences in the adjacent regions of the Carpathian area reached about the same values both in Poland and Slovakia. Maximum values were recorded in both countries in 2001, followed by a significant decrease which was probably due to climatic changes influencing egg survival. The year 2003, when the largest decrease in *E. multilocularis* occurred in Slovakia, was characterized by specific climatic factors, and in particular the lack of rainfall as reported by the Slovak Hydrometeorological Institute in Košice. Precipitation values were the lowest since the beginning of the 20th century (Sekáčová *et al.*, 2004), resulting in an increase in the desiccation of tapeworm oncospheres and a decrease in the population of voles which act as the main intermediate hosts of *E. multilocularis*. The relative density of small mammal populations in the surveyed areas of eastern Slovakia decreased from 120.6 exemplars per 100 tracks in 2001 to 32.9 exemplars per 100 tracks in 2003 (Stanko *et al.*, 2005).

The outcome of a 4-year study shows that prevalence and worm burden rates in both countries significantly increase towards the common state border and are higher in the border regions than in adjacent areas. These differences are due to geomorphological and ecological factors, which are crucial for the survival of tapeworm eggs and the subsequent spread of infection. It follows that as eggs of *E. multilocularis* are dry and high temperature-sensitive (Veit *et al.*, 1995), the tapeworm is likely to occur in a colder climates and its distribution range is limited by temperature and rainfall, and this also applies at the regional level.

Long-term epidemiological surveys have revealed, also in Slovak conditions, that the highest prevalence and infection rates occur in the areas with low mean annual air temperatures, high mean annual rainfall, high humidities and low temperatures in an active soil surface. These ecological factors, together with the landscape patterns and their exploitation also influence the survival of appropriate definitive and intermediate hosts. These specific climatic conditions are characteristic for the

Table 4. Mean worm burdens of *Echinococcus multilocularis* and percentage of red foxes infected in each category from Zone I + II in Poland and Slovakia, 2001–2004.

	Worm burden categories				
Country and zones	Low (1–10) %	Medium (11–100) %	High (101–1000) %	Very high (>1000) %	Mean worm burdens
Poland					
Zone I	22.9	41.5	23.7	11.9	332
Zone II	24.0	36.0	24.0	16.0	300
Total	36.2	36.2	15.5	12.1	322
Slovakia					
Zone I	14.5	33.9	29.0	22.6	1026
Zone II	19.0	38.1	26.2	16.7	659
Total	16.4	35.5	27.9	20.2	878

Carpathian regions of northeast Slovakia and southeast Poland.

Acknowledgements

The authors wish to thank the State Veterinary and Food Institutes in Prešov and Košice and Veterinary Inspectorate of Carpathian Foothills Voivodship in Krosno for sample collection. The investigation was supported by the project SPVV 51/028 08 00/028 0803 and by the Science Grant Agency VEGA 2/4179/24. The experiments performed comply with the current laws of Poland and the Slovak Republic.

References

Anon. (1994) *Statistical yearbook of the Slovak Republic 1993*. Bratislava, VEDA.

Anon. (1997) *Statistical yearbook of the Slovak Republic 1996*. Bratislava, VEDA.

Anon. (2003) *Statistical yearbook of the Slovak Republic 2002*. Bratislava, VEDA.

Baruš, V. (1961) Contribution to the knowledge of the helminthofauna in the wolf and wild cat in Czechoslovakia. *Československá Parazitologie* **8**, 11–14.

Dubinský, P., Svobodová, V., Turčeková, L., Literák, I., Martínek, K., Reiterová, K., Kolárová, L., Klimeš, J. & Mrlík, V. (1999) *Echinococcus multilocularis* in Slovak Republic: the first record in red foxes (*Vulpes vulpes*). *Helminthologia* **36**, 105–110.

Kinčeková, J., Auer, H., Reiterová, K., Dubinský, P., Szilágyová, M. & Lauko, L. (2001) The first case of autochtonous human alveolar echinococcosis in the Slovak Republic (case report). *Mitteilungen der Osterreichischen Gesellschaft für Tropenmedizin und Parasitologie* **23**, 33–38.

Kinčeková, J., Reiterová, K., Dubinský, P., Szilágyová, M., Johannes, R. & Gottas, M. (2002) A second case of autochtonous human alveolar echinococcosis in the Slovak Republic. *Helminthologia* **39**, 193–196.

Kinčeková, J., Dubinský, P., Dvorožňáková, E., Auer, H., Hudáčková, J., Daňová, M., Stanislayová, M. & Straka, L. (2005) Occurrence, classification and diagnostics of human alveolar echinococcosis in Slovakia. *Česká a Slovenská Gastroenterológie a Hepatológie* **59**, 11–16.

Malczewski, A., Rocki, B., Ramisz, A. & Eckert, J. (1995) *Echinococcus multilocularis* (Cestoda), the causative agent of alveolar echinococcosis in humans: first record in Poland. *Journal of Parasitology* **81**, 318–321.

Malczewski, A., Ramisz, A., Rocki, B., Bieňko, R., Balicka-Ramisz, A. & Eckert, J. (1999) *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Poland: an update of the epidemiological situation. *Acta Parasitologica* **44**, 68–72.

Miterpáková, M., Dubinský, P., Reiterová, K., Machková, N., Várady, M. & Šnábel, V. (2003) Spatial and temporal analysis of the *Echinococcus multilocularis* occurrence in the Slovak Republic. *Helminthologia* **40**, 217–226.

Mituch, J. (1963) Contribution to the knowledge of the helminthofauna in the wolf (*Canis lupus lupus* L.) in Czechoslovakia. *Československá Parazitológie* **10**, 119–123.

Mituch, J. (1964) On the helminth fauna of Felidae in Slovakia. *Helminthologia* **5**, 125–134.

Mituch, J. (1972) The helminthofauna of Carnivora in Slovakia and Czechoslovakia. *Folia Venatoria* **2**, 161–172.

Mituch, J., Hovorka, J., Hovorka, I. & Világiová, I. (1992) Helminths of carnivores on the model territory of the Hihg Tatras National Park. *Folia Venatoria* **22**, 191–200.

Prokopič, J. (1965) The helminthofauna of Carnivora in Czechoslovakia. *Československá Parazitológie* **12**, 207–226.

Raoul, F., Deplazes, P., Nonaka, N., Piarroux, R., Vuitton, D.A. & Giraudoux, P. (2001) Assessment of the epidemiological status of *Echinococcus multilocularis* in foxes in France using ELISA coprotests on fox faeces collected in the field. *International Journal for Parasitology* **31**, 1579–1588.

Sekáčová, Z., Šťastný, P., Faško, P. & Lapin, M. (2004) Extreme weather manifestation in 2003 in Slovak Republic. pp. 49–52 *in* Rožnovský, J. & Litschmann, T. (*Eds*) *Workshop on extremes of weather and climate*. Czech Republic, Brno.

Stanko, M., Mošanský, L., Fričová, J., Schniererová, E. & Čisláková, L. (2005) Long-term survey of natural focus in the East Slovakia. 27 pp. *Proceedings of the International Conference Zoonoses, their Agents and Vectors*, 4–6 April 2005, Smolenice, Slovakia.

STATSOFT, Inc. (2001) *Statistica, version 6*. http://www.statsoft.com.

Veit, P., Bilger, B., Schad, V., Schäfer, J., Frank, W. & Lucius, R. (1995) Influence of environmental factors on the infectivity of *Echinococcus multilocularis* eggs. *Parasitology* **110**, 79–86.

(Accepted 3 March 2006)