Journal of Helminthology (2001) **75,** 61–66 DOI: 10.1079/JOH200038

Echinococcus multilocularis in carnivores from the Klatovy district of the Czech Republic

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Abstract

A unique human case of alveolar echinococcosis was described in 1979 from the Klatovy district of the Czech Republic. However, there were no previous epidemiological studies in this area focusing on detection of the source of infection – *Echinococcus multilocularis* adults producing eggs. During the period June 1997 to April 1999, 29 out of a total of 46 (63.3%) red foxes (*Vulpes vulpes*) in the Klatovy district and one of four foxes (25.0%) in the Pilsen South district were found to be infected with adult worms of *E. multilocularis*. No *E. multilocularis* adults were found in other animals from the Klatovy district (i.e. three specimens of *Martes martes*, two *Martes foina*, one *Mustela erminea*, two *Meles meles* and one *Felis catus* f. *domestica*). An examination of faecal samples from 55 dogs (*Canis familiaris*) from the Klatovy district resulted in the detection of *E. multilocularis* DNA in one (1.8%) sample. The present results support the possibility that human alveolar echinococcosis previously described in the Czech Republic had the character of an autochthonous infection. There are also indications of a potential risk of infection to humans.

Introduction

The life cycle of the tapeworm *Echinococcus multilocularis* Leuckart, 1863 (Cestoda: Taeniidae) includes carnivores (mainly foxes) and small mammals (mainly rodents) as definitive and intermediate hosts respectively. Adult worms (2–4 mm long) live in the small intestine of the definitive hosts, where they reach sexual maturity and produce eggs. These eggs pass in the host faeces, most of them are fully embryonated, each with a developed larva (oncosphere), and infective to a suitable intermediate host. When ingested, viable eggs hatch in the stomach and small intestine and the oncosphere migrates and develops by asexual proliferation mostly in the host liver (Thompson, 1995).

Humans can be infected by accidental ingestion of *E. multilocularis* eggs. On rare occasions, infections in humans can develop into a potentially fatal disease. For assessing the risk of infection with this cestode in humans, knowledge on risk areas, potential sources, ways and extent of infection are required (Eckert & Deplazes, 1999).

A reliable technique for detection of parasites in definitive hosts is a parasitological examination of the small intestine at necropsy. However, recent new developments in faecal antigen and DNA detection for the diagnosis of intestinal infection with *E. multilocularis* (e.g. Dinkel *et al.*, 1998; Deplazes *et al.*, 1999) have increased our knowledge about the distribution of the cestode based on observations in e.g. The Netherlands and

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Slovak Republic (Dubinský *et al.*, 1999; van der Giessen *et al.*, 1999).

As recently as 1990, the distribution of *E. multilocularis* was largely restricted to eastern France, southern Germany, Switzerland and Austria (Rausch, 1995). However, during the last decade the range of cestode detection has spread, mainly in red foxes (*Vulpes vulpes*) in which *E. multilocularis* has been found in most parts of Germany, Belgium, Luxembourg, Liechtenstein, Poland, Czech Republic, Slovak Republic and The Netherlands (Eckert, 1997; Eckert & Deplazes, 1999; Kolářová, 1999; Romig *et al.*, 1999). Cats and dogs were also found to be naturally infected with adult *E. multilocularis* although in most endemic areas foxes are thought to be the main source of environmental contamination with eggs (Deplazes *et al.*, 1997).

In the Czech Republic, *E. multilocularis* adults in foxes were first discovered in 1995 in areas closely adjacent to Germany and Austria (Čada & Huml, 1996; Kolářová *et al.*, 1996; Pavlásek *et al.*, 1996) and subsequently Pavlásek *et al.* (1997) and Pavlásek (1998) reported this cestode mainly in red foxes in many areas of the country. Čada *et al.* (1999) described the finding of *E. multilocularis* in the domestic cat (*Felis catus* f. *domestica*) from the Tachov district (d.).

Šlais *et al.* (1979) described the first and still unique case of human alveolar echinococcosis, following *post-mortem* examination of a 74-year-old woman from Strážov, Klatovy d. This was followed, almost 20 years later, by the detection of the metacestode of *E. multilocularis* in the bank vole (*Clethrionomys glareolus*) by Martínek *et al.* (1998). However, as no previous study had focused on the infection risk of *E. multilocularis* to humans in this territory, the aim of the present study was to examine a range of carnivores, as potential sources of *E. multilocularis* infections.

Material and methods

Study sites

The majority of red foxes, some of the domestic dog faecal samples and all mustelids originated from the hunting ground (h.g.) of Hartmanice, located in the southern part of Klatovy d., West Bohemia, Czech Republic (49°10′, 13°27′). The area covers 17 km^2 and is located between 520 and 780 m above sea level. Fields, meadows and pastures account for 73.5% of the territory, forests 24.6%, built-up area (villages) 1.8% and water area 0.1%.

In Hartmanice h.g. a large increase in the fox population was recorded during the period of 1979–1998 (Anděra & Červený, 1994; Anděra & Červený, 1999 unpublished results) (fig. 1). The remainder of the foxes examined in the present study originated from other hunting grounds of the Klatovy d. and from the Pilsen South d. (table 1).

Collection of samples

During the period of June 1997 to April 1999, a total of 50 red foxes (*V. vulpes*) were examined: 30 from Hartmanice h.g., 16 from other parts of the Klatovy d. and four from the Pilsen-South d. (table 1). Three pine martens (*Martes martes*), two stone martens (*Martes foina*), one stoat (*Mustela erminea*), two badgers (*Meles meles*) and one domestic cat (*Felis catus* f. *domestica*) from Hartmanice h.g. were also examined. The majority of the animals were shot by authorized hunters; the domestic cat, stoat and badgers were the victims of motor vehicle accidents. Each intestine was removed as soon as possible and kept at −5°C before being transported to the laboratory for examination. Following Veit *et al.* (1995), all specimens were deep frozen (−80°C) for at least 48 h for inactivation of *E. multilocularis* eggs.

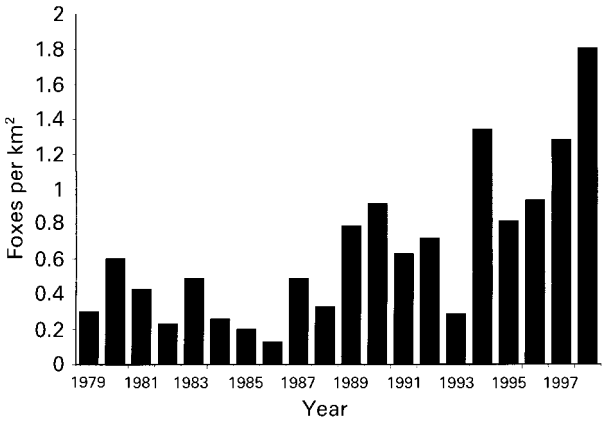

Fig. 1. The red fox population from the hunting ground of Hartmanice during 1979–1998 based on yearly hunting bags.

Faecal samples from 55 dogs were collected during June 1998 to October 1998: 14 originating from Hartmanice h.g. and 41 from northern areas of the Klatovy d. Faeces were either stored frozen at −20°C or fixed in 80% ethanol.

Examination of samples

Each small intestine was cut into 10 cm sections which were opened longitudinally. Using an Olympus SZ-60 stereomicroscope, the mucosa and gut content were carefully observed in a Petri dish containing water. If *E. multilocularis* were not found, a scraping method (Eckert *et al.*, 1984) was subsequently used to locate the parasites. A minimum of seven mucosal smears were taken from different locations in the lower part of the small intestine, using microscope slides and the squashed tissue examined under the stereomicroscope. When found, adult worms of *E. multilocularis* were collected and counted.

Mean prevalences were calculated for all studied areas, with confidence intervals (CI_{95}) (alternative distribution, 95% probability) being determined after Hátle & Likeš (1972). The intensity of infection was classified after Ewald (1993) into three ranges: low (1–100 parasites), medium (101–1000), and high (>1000). Although the age and sex of animals were registered, the data are not presented due to the low and thus statistically not significant number.

Dog faeces were examined for presence of an *E. multilocularis* specific DNA sequence using a nested

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Table 1. The proportion of foxes infected with *Echinococcus multilocularis* in the Klatovy and Pilsen South districts from June 1997 to April 1999.

Region	Foxes examined	Foxes infected	Proportion of foxes infected (%)[a]	Proportion of foxes infected (%)[b]		
				low	medium	high
Hartmanice hunting ground, Klatovy district	30	17	56.7 (39.2–72.6)	35	59	6
Northen part of Klatovy district	16	12	75.0 (50.5–89.8)	42	33	25
Klatovy district (total)	46	29	63.3 (48.6–75.5)	38	48	14
Pilsen South district	4	1	25.0 (4.6–69.9)	100	0	0
Total	50	30	60.0 (48.7–66.2)	40	47	13

[a] Confidence intervals in 95% probability level, % range in parentheses.
[b] Intensity of infection: low, 1–100 parasites(medium, 101–1000 parasites, high, 1001–5000 parasites.

polymerase chain reaction (PCR) technique after Dinkel *et al.* (1998). A total of 10 µl of PCR products was separated on to a 1.5% agrose gel and stained with ethidium bromide.

Results

Adult worms of *E. multilocularis* were detected in the small intestine of 30 of 50 foxes (prevalence 60.0%, CI_{95} 48.7–66.2%) from all localities. In the Klatovy d., 29 of 46 foxes were positive (63.3%, CI_{95} 48.6–75.5%) and in this region the majority of infected foxes originated from Hartmanice h.g., where 17 of 30 foxes (56.7%, CI_{95} 39.2–72.6%) were positive. One of four foxes (25.0%, CI_{95} 4.6–69.9%) from the Pilsen-South d. were infected.

A study of the spatial distribution of foxes in Hartmanice h.g. showed that the majority of infected foxes (13 of 17 specimens, 76.5%), inhabited an open landscape, whilst most of the uninfected foxes, 11 of 13 specimens (84.6%) were shot near large forest complexes (fig. 2).

Worm burdens varied between one and 5000 worms per fox. Of the 30 foxes infected (table 1), infection intensities were low in 40% (12 foxes), medium in 47% (14 foxes) and high in 13% (four foxes). Adults of *E. multilocularis* were found in various stages of maturation. Worms with gravid proglottids were found in 27 of 30 (90.0%) samples, whereas in the remaining three samples early segmentation stages (one fox) or mature but sterile worms (two foxes) were observed. In one fox, mature adults of *E. multilocularis* as well as evaginated protoscoleces occurred simultaneously.

The remaining carnivore species (two *Martes martes*, two *Martes foina*, one *Mustela erminea*, one *Meles meles* and one *Felis catus* f. *domestica*) were negative, although one of 55 samples of dog faeces (1.8%) from the town of Hartmanice was found to be positive. Agarose gel electrophoresis of the PCR products of this sample showed the 250 bp DNA band indicating the presence of *E. multilocularis* DNA (fig. 3).

Discussion

Since 1994 many red foxes have been examined for the presence of *E. multilocularis* in the Czech Republic (e.g. Čada & Huml, 1996; Pavlásek, 1998). To date, no previous study has compared the occurrence of both adult and larval stages in carnivores from the same geographical area. The present study, therefore, focused on the examination of carnivores in the Klatovy d., an area characterized by the discovery of larval stages of *E. multilocularis* in rodents (Martínek *et al.*, 1998) and also by the detection of a unique Czech case of human alveolar echinococcosis (Šlais *et al.*, 1979).

Recent European data indicate that red foxes are the main definitive hosts for *E. multilocularis* (Deplazes *et al.*, 1997) and prevalence values reach up to 60% in certain European areas (e.g. Schantz *et al.*, 1995). Pavlásek (1998) recorded mean prevalences of 2.5 to 22.9% in foxes from five Czech regions, with the most affected being adjacent to Germany and Austria, mainly in South and West Bohemia. The prevalence in West Bohemian districts (Cheb, Karlovy Vary) varied between 25.8 and 30.8%. Čada & Huml (1996) had previously reported a prevalence of 2% in the same area (Karlovy Vary d.) and 8% in foxes from the Domažlice d. The present results indicate a prevalence of 60% in a closely adjacent area, suggesting a higher population density of susceptible definitive and intermediate hosts. The spatial distribution of foxes examined in the Hartmanice area showed that most infected foxes (76.5%) came from an open landscape, while most of the uninfected foxes (84.6%) were shot near large forest complexes (fig. 2). This is likely to be due to the presence of the intermediate host of *E. multilocularis*, the common vole (*Microtus arvalis*), the density of which is higher in agrocoenoses than in forest complexes (Červený, 1989; Anděra & Červený, 1994).

Using a nested PCR technique, specific *E. multilocularis* DNA was detected in a dog sample from the town of Hartmanice, and this underlines the need to focus on molecular studies on *E. multilocularis* in carnivores in close contact with humans, especially in urban areas.

The absence of *E. multilocularis* in mustelids confirms the findings of Zeyhle *et al.* (1990) that these carnivores do not serve as definitive hosts. It is of interest to note that even when the prevalence of *E. multilocularis* is high in some European areas (Schantz *et al.*, 1995), the incidence of human infections persists at a low level, e.g. the mean annual incidence rates of alveolar echinococcosis vary between 0.02 and 1.4 per 100,000

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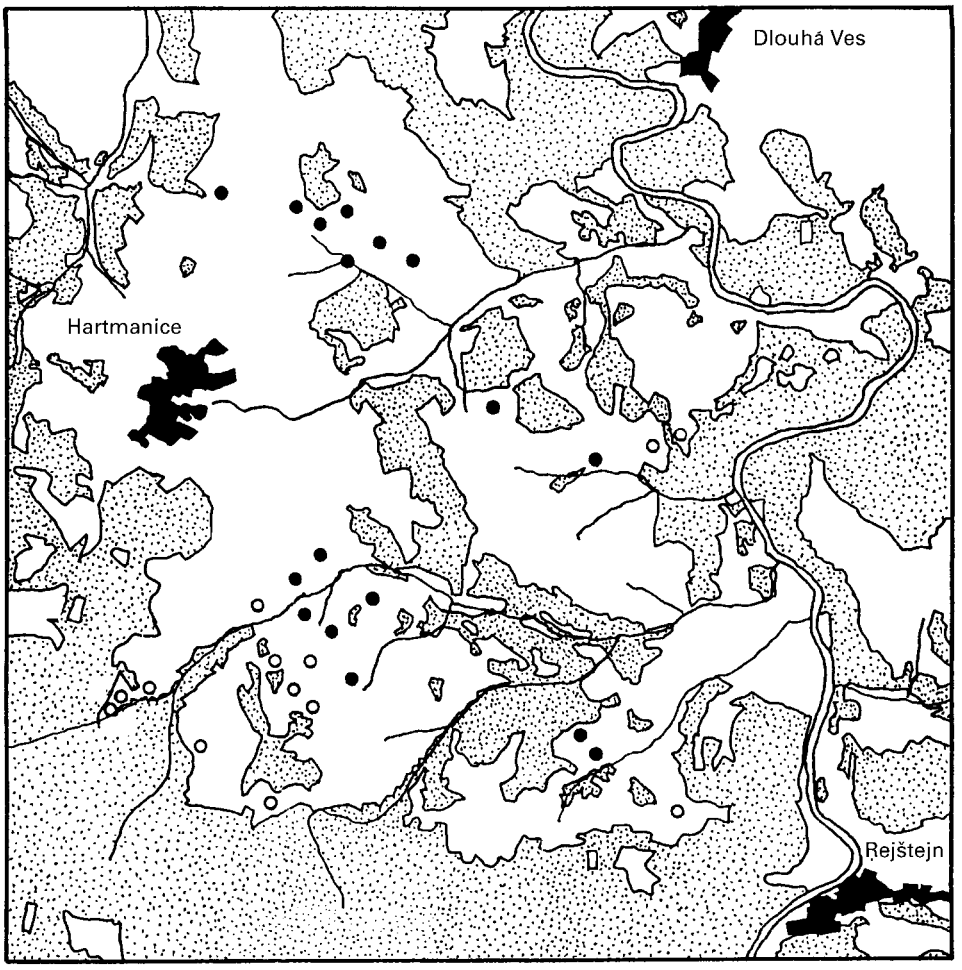

Fig. 2. Spatial distribution of foxes infected with *Echinococcus multilocularis* (●) and uninfected foxes (○) from the hunting ground of Hartmanice in the Klatovy district. Clear and dotted areas represent open landscape and forest complexes, respectively.

inhabitants in central European endemic areas (Eckert & Deplazes, 1999). The modes of transmission to humans are known but not in detail (Eckert, 1997), and hence the occurrence of *E. multilocularis* in carnivores from new areas of the Czech Republic underlines the necessity for assessing the risk of infection to human populations.

As in the present study, the majority of data reporting the occurrence of *E. multilocularis* in new European areas (Belgium, Liechtenstein, Poland, Czech Republic, Slovak Republic, The Netherlands) are from the 1990s (Brochier *et al.*, 1992; Ewald & Eckert, 1993; Malczewski *et al.*, 1995; Kolářová *et al.*, 1996; Dubinský *et al.*, 1999; van der Giessen *et al.*, 1999) and therefore it is not possible to confirm whether this cestode species spread from historically known endemic European foci to new areas. However, the presence of adult *E. multilocularis* would be assumed to be present considerably earlier than in the 1990s, as intermediate and definitive hosts occur throughout the whole of Europe (Mitchell-Jones *et al.*, 1999). Moreover, larval stages of *E. multilocularis* were found in the intermediate hosts many years before the detection of the adult stages, e.g. in the Czech Republic human alveolar echinococcosis was reported by Šlais *et al.* (1979) approximately 15 years prior to detection of the first positive fox. Removal of the artificial barrier in 1990 between the Czech Republic and neighbouring territories of western Europe probably had no substantial influence on the spread of foxes infected with *E. multilocularis* from German and Austrian endemic foci. A comparison of data from Malczewski *et al.* (1995) and unpublished results of Blaha & Bzdil (1996, personal communication) from the Czech Republic showed that at the same time as positive foxes were detected in the western Czech areas (e.g. Pavlásek, 1998; Čada & Huml, 1996), *E. multilocularis* adults were reported in the southern part of Poland and from the eastern part of the Czech Republic (Opava d.). It seems that the small adults of *E. multilocularis* might have escaped the attention of parasitologists, mainly in cases where the prevalence of infection was low.

The detection of *E. multilocularis* in foxes and dogs in the Czech Republic underlines the need for assessing the

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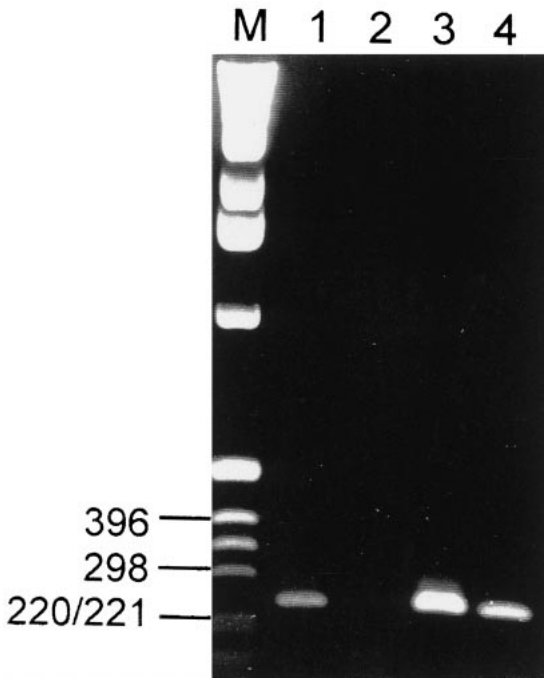

Fig. 3. Nested PCR. Lane 1, DNA from faecal sample of *Echinococcus multilocularis* infected dog; lane 2, negative control; lane 3, DNA extracted from *E. multilocularis* (Czech origin); lane 4, *E. multilocularis* eggs; lane M, molecular weight marker X (Boehringer Mannheim). 250 bp band visible in lanes 1, 3 and 4.

risk of infection to humans and for the continuing examination of carnivores, particularly those living in close contact with humans. The presence of adult *E. multilocularis* in West Bohemia showed that the previously described, but still unique, case of human alveolar echinococcosis should have an autochthonous character of the disease in the past and, moreover, indicates a potential risk of infection to humans at the present time, especially as the fox population appears to be increasing both in the Czech Republic (Anděra & Červený, 1994) and other European countries (Lucius & Bilger, 1995).

Acknowledgements

This investigation was supported by the Grant of the Ministry of Health of the Czech republic No. 4962-3. We are most grateful to all who assisted in collecting data in field, namely: MVDr Ludvík Mikoška (Sušice), MVDr Václav Špeta (Blatná), MVDr Josef Nejdl (Klatovy) and Josef Krutský (Hořejší Krušec).

References

Anděra, M. & Červený, J. (1994) Atlas of distribution of the mammals of the Šumava Mts. region (SW-Bohemia). *Acta Scientiarum Naturalium Academiae Scientarium Bohemicae Brno* **28**, 1–111.

Brochier, B., Coppens, P., Losson, P., Aubert, M.F.A., Bauduin, B., Barrat, M.J., Costy, F., Peharpre, D., Pouplard, L. & Pastoret, P.P. (1992) Enquęte sur l'infestation du renard rouf (*Vulpes vulpes*), par *Echinococcus multilocularis* en province de Luxembourg (Belgique). *Annales de Médicine Véterinaire* **136**, 497–501.

Čada, F., Martínek, K. & Kolářová, L. (1999) Domestic cat (*Felis catus f. domestica*) as the final host of *Echinococcus multilocularis* tapeworm. *Veterinářství* **49**, 2–3.

Čada, F. & Huml, O. (1996) *Echinococcus multilocularis* in red foxes from West-Bohemian region in 1995. *Veterinářství* **4**, 160–162.

Červený, J. (1989) Contribution to the small mammal fauna (Insectivora, Rodentia) of West Šumava mountains. *Lynx* **25**, 5–28.

Deplazes, P., Mathis, A. & Eckert, J. (1997) Diagnosis of *Echinococcus* infection in the final host by coproantigen and DNA detection. *Archivos Internationales de la Hydatidosis* **32**, 198–201.

Deplazes, D., Alther, P., Tanner, I., Thompson, R.C.A. & Eckert, J. (1999) *Echinococcus multilocularis* coproantigen detection by enzyme-linked immunosorbent assay in fox, dog and cat populations. *Journal of Parasitology* **85**, 115–121.

Dinkel, A., Nickisch-Rosenegk, von, M., Bilger, B., Merli, M., Lucius, R. & Romig, T. (1998) *Echinococcus multilocularis* in the definitive hosts: coprodiagnosis by PCR is an alternative to necropsy. *Journal of Clinical Microbiology* **36**, 1871–1876.

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

Eckert, J. (1997) Epidemiology of *Echinococcus multilocularis* and *E. granulosus* in Central Europe. *Parassitologia* **39**, 337–344.

Eckert, J. & Deplazes, P. (1999) Alveolar echinococcosis in humans: the current situation in central Europe and the need for countermeasures. *Parasitology Today* **15**, 315–319.

Eckert, J., Gemmell, M.A., Matyas, Z. & Soulsby, E.J.L. (1984) *Guidelines for surveilance, prevention and control of echinococcosis/hydatidosis.* 2nd edn, Geneva, WHO.

Ewald, D. (1993) Prävalenz von *Echinococcus multilocularis* bei Rotfüchsen in der Nord-, Ost-, und Südschweiz sowie im Fürstentum Liechtenstein. 110 pp. Dissertation, Phil. II, Universität Zürich

Ewald, D. & Eckert, J. (1993) Verbreitung und Haufigkeit von *Echinococcus multilocularis* bei Rotfüchsen in der Nord-, Ost-, und Südschweiz sowie im Fürstentum Liechtenstein. *Zeitschrift für Jagdwissenschaft* **39**, 171–180.

Giessen, J.W.B., van der Rombout, Y.B., Franchimont, J.H., Limper, L.P. & Homan, W.L. (1999) Detection of *Echinococcus multilocularis* in foxes in The Netherlands. *Veterinary Parasitology* **82**, 49–57.

Hátle, J. & Likeš, J. (1972) *Basis of probability counting and mathematical statistics.* Bratislava, SNTL.

Kolářová, L. (1999) *Echinococcus multilocularis* – new epidemiological insights in Central and Eastern Europe. *Helminthologia* **36**, 193–200.

Kolářová, L., Pavlásek, I. & Chalupský, J. (1996)

Echinococcus multilocularis Leuckart, 1863 in the Czech Republic. *Helminthologia* **33**, 59–65.

Lucius, R. & Bilger, B. (1995) *Echinococcus multilocularis* in Germany: increased awareness or spreading of a parasite? *Parasitology Today* **11**, 430–434.

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.

Martínek, K., Kolářová, L., Červený, J. & Andreas, M. (1998) *Echinococcus multilocularis* (Cestoda: Taeniidae) in the Czech Republic: the first detection of metacestodes in a naturally infected rodent. *Folia Parasitologica* **45**, 332–333.

Mitchell-Jones, A.J., Amori, G., Bogdanowitcz, W., Kruštufek, B., Reijnders, P.J.H., Spitzenberger, E., Stubbe, M., Thissen, J.B.H., Vohralík, V. & Zima, J. (1999) *The atlas of European mammals.* London, Poyser.

Pavlásek, I. (1998) Actual situation in the occurence of *Echinococcus multilocularis* in red foxes in Europe and the Czech Republic. *Remedia-Klinická Mikrobiologie* **2**, 233–240.

Pavlásek, I., Chalupský, J. & Kolářová, L. (1996) *Echinococcus multilocularis* – the less known tapeworm from foxes. *Veterinářství* **46**, 164–167.

Pavlásek, I., Chalupský, J., Kolářová, L., Horyna, B. & Ritter, J. (1997) Incidence of *Echinococcus multilocularis* Leuckart, 1863 in foxes (*Vulpes vulpes*) in the Czech Republic. *Epidemiologie Microbiologie Immunologie* **46**, 158–162.

Rausch, R.L. (1995) Life cycle patterns and geographic distribution of *Echinococcus* species. pp. 89–134 *in* Thompson, R.C.A. & Lymbery, A.J. (*Eds*) *Echinococcus and hydatid disease.* Wallingford, CAB International.

Romig, T., Bilger, B., Dinkel, A., Merli, M. & Mackenstedt, U. (1999) *Echinococcus multilocularis* in animal hosts: new data from western Europe. *Helminthologia* **36**, 185–191.

Schantz, P.M., Chai, J., Craig, P.S., Eckert, J. & Jenkins, D.J. (1995) Epidemiology and control of hydatid disease. pp. 233–354 *in* Thompson, R.C.A. & Lymbery, A.J. (*Eds*) *Echinococcus and hydatid disease.* Wallingford, CAB International.

Šlais, J., Mádle, A., Vanka, K., Jelínek, F., Černík, V., Průchová, M. & Jindra, J. (1979) Alveolar hydatidosis (echinococcosis) diagnosed by liver puncture biopsy. *Časopis Lékařů Českých* **118**, 472–475.

Thompson, R.C.A. (1995) Biology and systematics of *Echinococcus*. pp. 1–50 *in* Thompson, R.C.A. & Lymbery, A.J. (*Eds*) *Echinococcus and hydatid disease.* Wallingford, CAB International.

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

Zeyhle, E., Abel, M. & Frank, W. (1990) Epidemiologische Untersuchen zum Vorkommen von *Echinococcus multilocularis* bei End- und Zwischenwirten in der Bundesrepublik Deutschland. *Mitteillungen der Österreichischen Gesellschaft für Tropenmedizin und Parasitologie* **12**, 221–232.

(Accepted 24 July 2000)