

## Prace oryginalne

# Analysis of the epidemiological factors influencing vulpine trichinellosis in ecologically different regions of Slovakia

Zuzana Hurníková, Danica Bartková and Pavol Dubinský

Parasitological Institute of the Slovak Academy of Sciences, Košice, Slovak Republic

Corresponding author: Zuzanna Hurníková, Parasitological Institute, Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovak Republic; E-mail: hurnikz@saske.sk

**ABSTRACT. Introduction.** In the Slovak Republic, trichinellosis circulates almost exclusively in the sylvatic cycle, with main reservoir host red fox and wild boar and sporadic occurrence of human outbreaks. A detailed study was performed in five ecologically different regions of eastern Slovakia with more profound regard to eco-geographical and anthropogenic influences to natural fox habitat. **Material and methods.** In total of 689 red foxes (*Vulpes vulpes*) hunted in selected regions in 2005/2006 was examined using artificial digestion method. Larvae obtained from infected samples were on the species level characterised using multiplex PCR analysis. **Results.** The study revealed a total prevalence of 15.6%, with most frequent occurrence of infected foxes in the mountain of the Volovské Vrchy (25.2%) where both human habitation and fox population are very dense. High prevalence rates were found in the Košická Kotlina Basin (19.6%) with urbanised landscape, concentrated human activities and low fox population and in national park of the High Tatras (15.8%) where the inhabitants and fox population are relatively low. In the remote localities of the Nízke Beskydy Highlands that represent ideal fox habitat free of any human impact, 14.2% of foxes harboured *Trichinella* larvae. The lowest occurrence of infected foxes (6.9%) was found in agrarian areas of the Východoslovenská Nížina Lowland, with relatively low inhabitants and fox population density. In all localities *Trichinella britovi* was the most important etiological agent of sylvatic trichinellosis.

**Key words:** Slovakia, trichinellosis, *Vulpes vulpes*.

## Introduction

Natural ecosystem plays an important role in the maintenance of the sylvatic trichinellosis in the nature and human behaviour influences the ecology of trichinellosis in both, domestic and sylvatic habitat. As ecosystem become more fragmented and human — wildlife interactions intensify, a clear understanding of what constitutes a truly natural distribution of *Trichinella* genotypes and species is needed. Foxes are frequently reported as harbouring trichinae, but they are among few predators that adapt well to human habitation and are still numerous in many parts of the world. This implies potential for the urban *Trichinella* transmission. Elevated populations of small carnivorous mammals and increased interactions of these populations with humans and their domestic animals eventuate, that trichinellosis from wildlife sources appears sporadi-

cally in human populations.

In the Slovak Republic trichinellosis in sylvatic cycle is maintained in particular in red fox and wild boar as main reservoirs and *T. britovi* as predominant species. The substantial increase of red fox population in recent provides a potential for spread of this parasite in wildlife. Domestic cycle did not operate in the Slovak territory for many decades, although the risk for acquiring trichinellosis particularly from game meat continues to be a threat in our region. *Trichinella spiralis* has been present in the past in domestic and synanthropic animals, but currently is circulating only in the sylvatic cycle as a residuum of its former presence in pigs. The results of longitudinal study (2000-2005) pointed substantial increase of *Trichinella* prevalence in foxes from 4.9% in 2000 up to 13.3% in 2005 together with geographical stratification of occurrence [1]. The objective of our study was to illuminate potential

interacting factors affecting the epidemiology of trichinellosis with more profound regard to eco-geographical and anthropogenic influences on natural fox habitat. The zoogeographical, climatic, and landscape characteristics and several parameters with respect to human impact on the fox habitat-human population density, the intensity of human activity and type of human intervention to the country, were followed.

### Materials and methods

The study was performed on 689 red foxes (*Vulpes vulpes*) originating from five ecologically different regions of eastern Slovakia. Respective regions are marked on Fig. 1 and their characteristics are listed in Table 1. Foxes were hunted within the control program of antirabic vaccination in 2005-2006. Muscles samples were taken from upper part of front legs (*m. biceps brachii* and *m. triceps brachii*), which has been shown to be the predilection site for foxes [2]. A minimum of 15 g muscle tissue was examined individually by artificial HCl-pepsin digestion according to Kapel and Gamble [3]. Muscle samples were homogenised by blending and digested with digestive fluid (1 litre 45°C water +10 ml 37% HCl + 10 g pepsin 1:10 000 NF) on magnetic stirrers in an incubator at 45°C temperature up to 2 hours (until the digestion procedure was completed). Afterwards larvae were recovered by sedimentation in Pilsner glasses and counted in Petri

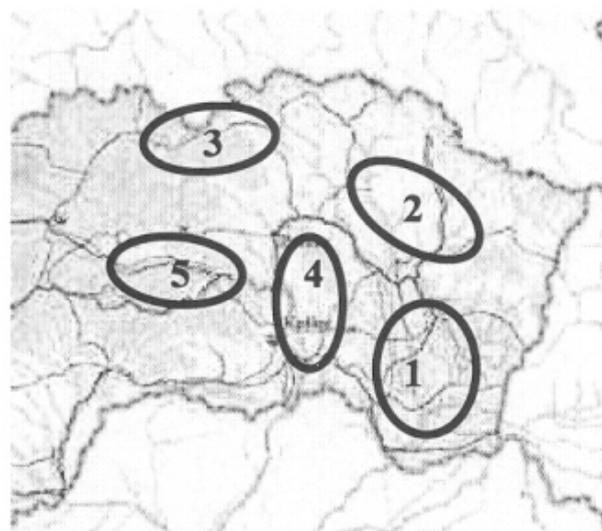


Fig. 1. Localisation of ecologically different areas under study

- 1 — Východoslovenská Nížina Lowland
- 2 — Nízke Beskydy Highlands
- 3 — High Tatras
- 4 — Košická Kotlina Basin
- 5 — Volovské Vrchy Mountain

dishes under a stereomicroscope. Larvae were stored in 96 % alcohol until molecular species identification. The intensity of infection was calculated as number larvae per gram of muscle tissue (LPG).

*Trichinella* larvae, collected from infected foxes, were identified at species level by the multiplex polymerase chain reaction (multiplex PCR).

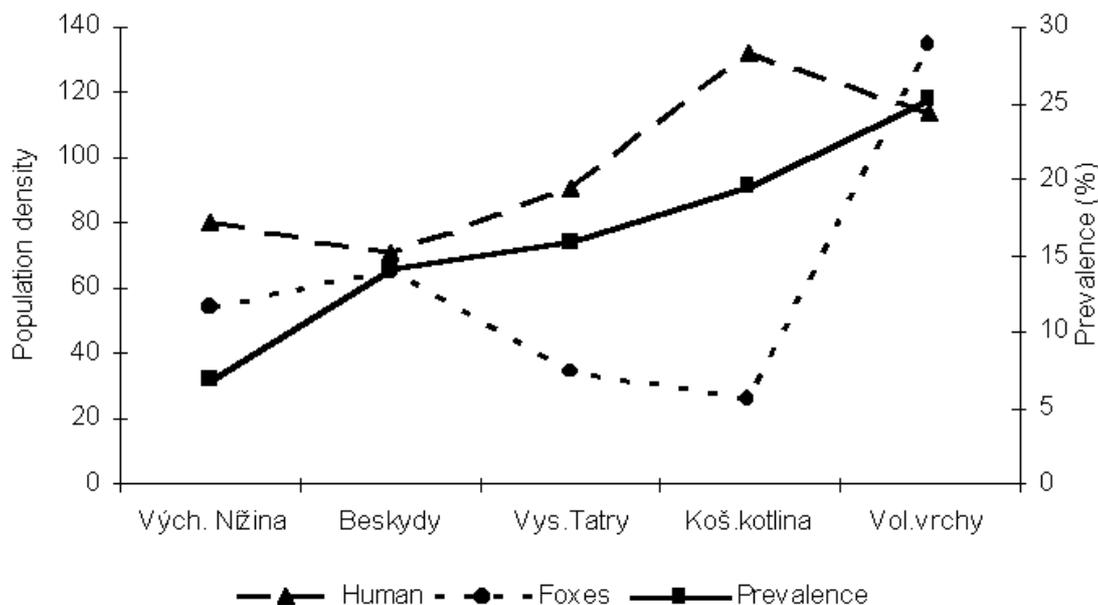


Fig. 2. The relations between the prevalence of vulpine trichinellosis, inhabitants' density (per km<sup>2</sup>) and fox population density (animals hunted per 1000 km<sup>2</sup>)

Table 1. The characteristics of different ecological regions potentially influencing the prevalence of vulpine trichinellosis

Locality	Landscape	Altitude	Average year temperature	Foxes hunted per 1000 km <sup>2</sup>	Human population per km <sup>2</sup>	Human interference in region	Prevalence (%)	LPG
Východoslovenská Nížina	lowland	90-110	9.5 °C	54	80.3	compact habitation, intensively exploited agricultural and arable land, foxes attracted by dumps and domestic animals	6.9	1.3
Nízke Beskydy	highlands	400-650	6 °C	65.5	70.4	national parks, protected and remote localities, forests, beech-woods, alps and meadows, free of any human impact, abundance of natural food sources	14.2	9.4
High Tatras	alpine mountain	700-2655	2.4 °C	34	90	national park, frequent tourism, extreme habitat for foxes	15.8	3.9
Košická Kotlina	basin	180-430	8.6 °C	26	131.5	urbanised landscape, concentrated human activities, agriculture, traffic, inadequate density of natural prey for foxes	19.6	5.7
Volovské Vrchy	mountain	300-1100	4 °C	135	113	dense settlements in lower parts, forested parts exploited for tourism, timber production, fox population overdispersed	25.2	11.1

Extraction of DNA and PCR amplification was done according to Zarlenga et al. [4] modified to a protocol used at the International *Trichinella* Reference Centre. The primer pairs were derived from expansion segment V (ESV) and internal transcribed spacers ITS1. The amplification products were separated on an agarose gel and stained with ethidium bromide before visualisation. All PCRs included negative and positive controls.

The positions of hunted foxes were located by cadastral number of the areas and the zoogeographical, climatic, and landscape characteristics with respect to human impact on the fox habitat-human population density, the intensity of human activity and type of human intervention to the country, were followed. Whereas quantitative data on red fox stock are not disposable since 1997, the density of population was estimated according to statistical data of hunting companies as number of captured animals per year.

## Results

Out of 689 red foxes inspected within the study 108 harboured *Trichinella* larvae that represents the total prevalence of 15.6%.

Vulpine trichinellosis was found to be most frequent in mountain areas of the Volovské Vrchy

where the prevalence rate was 25.2%. *Trichinella* larvae harboured 19.6% of foxes inhabiting the Košická Kotlina Basin, 15.8% of foxes captured in alpine mountain the High Tatras and prevalence 14.2% was found in foxes from the highlands of the Nízke Beskydy. Low prevalence of trichinellosis (6.9%) occurred in vulpine population of the Východoslovenská Nížina Lowland (Table 1).

Trichinellosis was most prevalent in localities with average altitude of 400-700 m above sea level (a.s.l) and in territories with high pressure of human activities. A correlation was found between the density of human population, the density of fox population and prevalence (Fig. 2). The evaluation of the relation between prevalence and intensity of infection in respective regions revealed strong dependence — the infection was most intensive in region with highest prevalence and lowest in locality with low prevalence rate.

In total 78 isolates from infected foxes were genotypically characterised by multiplex PCR protocol. The results revealed that *Trichinella britovi* is the most important etiological agent of sylvatic trichinellosis in all respective localities. All but 1 foxes harboured only *T. britovi* larvae, in one fox originating from south part of mountain Volovské Vrchy the mixed infection with *T. spiralis* and *T. britovi* was detected.

## Discussion

In Slovakia, as well as in most regions of Europe, red fox is the specific reservoir of *Trichinella* parasite in the sylvatic cycle. It is generally considered that sylvatic trichinellosis affects carnivores with cannibalistic and scavenger behaviour and higher population density causes intense competition for food and thus results in more scavenging and cannibalism, which are the major routes of *Trichinella* transmission [5]. In some countries of the European Union, cannibalism among foxes and landscape characteristics (mountains vs. lowlands) have been considered key factors in maintaining this parasite in nature [5, 6]. Currently, in the central and southern regions of the EU, sylvatic trichinellosis is most prevalent among foxes living either at the level of 400-500 m above the sea, or in protected areas, where the environment is less disturbed and the mountain climate (higher moisture, lower temperatures) favours the survival of muscle larvae in host carrion for a longer period of time [7]. Sylvatic trichinellosis is more widespread in the northern countries (Sweden and Finland, Norway). Lowlands are more likely to be *Trichinella*-free because of the greater impact of humans on the environment [8].

Evaluation of vulpine trichinellosis in five ecologically different regions of Slovakia showed that the parasite was most frequent in foxes hunted in the Volovské Vrchy Mountain, where the prevalence of disease reached 25.2%. This is an endemic mountain area with average altitude 700 m a.s.l., the population density of red fox is relatively high (135 foxes hunted per 1000 km<sup>2</sup>). An average density of inhabitants per km<sup>2</sup> is 113, human occupy mainly lower parts of mountain, where settlement is dense, and the higher, forested parts of hill-country are exploited for tourism, timber production and exploitation. In all likelihood elevated population density of foxes followed by reduction of their operating territories implied frequent cannibalism within fox population, which eventuated to increase of parasite prevalence.

In the contrary, in region of the Východoslovenská Nížina lowland, with high ratio of anthropogenic influence, the relatively low prevalence rate of 6.9% was found. The human habitation is very compact (80/km<sup>2</sup>) and the lowland with substantive portion of agricultural land is intensively exploited, with majority of arable land. Human impact markedly affected the fox habitat — in the landscape all natural brushwood was erased.

Thus the biotope becomes not sufficient for foxes and they are attracted by abundant human dumps and domestic animals and the risk of transmission of sylvatic trichinellosis due to cannibalistic fox behaviour decreased.

The attraction of human settlements for foxes is of importance in consideration of high prevalence rates found in foxes inhabiting the area of the Košická Kotlina Basin. Up to 19.6% of examined foxes were found to be positive for *Trichinella* — infection. Taking into account that in this region the degree of anthropogenic incidence is very high (more than 131 inhabitants/km<sup>2</sup>) and majority of the region is formed by urbanised landscape, the elevated population of urban foxes and incidence of infected foxes near by human population represents increased risk for formulation of domestic cycle or occurrence of human outbreaks. The human activity concentrates the landscape processes thus influencing the amplitudes in trophic niche of foxes. Cannibalism and fox-feeding behaviour, typical for remote and mountain areas, is now present also in this locality due to elevated concentration of fox population and inadequate density of natural prey.

In the area of the Nízke Beskydy Highlands with positions above sea 400-650 m (525), seven national parks and several protected localities are situated. The remote natural landscape reach in forests, beech woods, and mountain-meadows is free of any human impact and represents an ideal fox habitat. The relatively low (14.2%) prevalence rate in foxes inhabiting this region could to be explained by abundance of natural food sources and prey and foxes are not obliged to scavenging of fox carcasses and cannibalism. Very similar epidemiological situation was observed in the Vysoké Tatry. Entire alpine mountain is a national park area with elevation 700-2655 m a.s.l. The human settlements are localised in headland, human population reaches density of 90 inhabitants/km<sup>2</sup> and tourism is frequent. The fox population is not very high (34 foxes hunted per 1000 km<sup>2</sup>), as foxes usually avoid of extreme habitats. *Trichinella* prevalence of 15.8% was found within respective vulpine population.

The results of our study revealed, that trichinellosis in Slovakia is widespread not only in mountain areas but also in lowlands covered by broad-leaved forests and agricultural land and also in rural areas. This fact is probably caused by increase of red fox population 3-4 fold in last few years account on the sanitation due to the antirabic vaccination and in present the numbers of this carnivore is 7-8 times

over dispersed (270 foxes/1000 km<sup>2</sup>). The high population pressure has implied migration of foxes into the areas they didn't inhabit before. Also in consequence of reduction of operating territories the change of feeding behaviour appeared. The cannibalism and scavenger behaviour typical for wilderness and mountain areas, where no more attractive food sources are, is actual nowadays also in lower altitudes above sea level. The human impact on the environment contributes to the feeding habit changes by reduction of fox habitats and subsequently the reductions of population of typical prey (rodents, rabbits, birds, etc). This implies the food competition, cannibalism and scavenging of human garbage and domestic animals, representing an abundant food source for foxes living near to human habitats. Increase of number of infected foxes in urban areas represents the risk for creation of domestic cycle and emergence of human outbreaks.

Our finding revealing *Trichinella britovi* to be predominant species in examined territories with 98.8% of animals infected with this species are consistent with observations that *T. britovi* is the predominant species in the red fox population in Europe. The distribution of this species in the Slovak Republic is comparable to those observed in Poland (72.7% *T. britovi*) [9], Czech Republic [10], northern part of Hungary [11] and Ukraine [12]. Mixed infection found in fox (*T. spiralis* + *T. britovi*) is not surprising as mixed infections were already detected in wildlife [13-15]. The study of Pozio et al. [5] showed that the distribution of *Trichinella* among foxes is influenced by both environmental and human behaviour factors — also if regarding the different species of *Trichinella*.

Land use and the spatial organisation of human activities in the natural environment began to take on significance particularly in association with conflicts that have occurred in the landscape due to the human influence. Land cover integrated with georelief characteristics, provides the background for observing the intensity of landscape processes, changes in the landscape and identification of human influences on host biology. Therefore it is inevitable consider all potential interacting factors affecting the epidemiology and ecology of trichinellosis when analysing potential risk for human and designing effective preventive strategies.

### Acknowledgements

The study was supported by Slovak Grant Agency

VEGA 2/4179/26 and contract No. APVT-51-010704 and in part by the European project MED-VET-NET (WP11, Trichinet).

### References

- [1] Hurníková Z. 2004. Host-parasite-landscape interactions and their influence on *Trichinella* distribution in the Slovak Republic. *Programme and Abstracts on IX. European Multicolloquium of Parasitology, 18.-23.7. 2004, Valencia, Spain*: 373.
- [2] Kapel C.M.O., Henriksen S.S., Dietz H.H., Nansen P. 1994. A study on the predilection sites of *Trichinella spiralis* larvae in experimentally infected foxes (*Alopex lagopus*, *Vulpes vulpes*). *Acta Veterinaria Scandinavica* 35: 125-132.
- [3] Kapel C.M.O., Gamble H.R. 2000. Infectivity, persistence, and antibody response to domestic and sylvatic *Trichinella* spp. in experimentally infected pigs. *International Journal for Parasitology* 30: 215-221.
- [4] Zarlenga D.S., Chute M.B., Martin A., Kapel C.M.O. 1999. A multiplex PCR for unequivocal differentiation of six encapsulated and three non-encapsulated genotypes of *Trichinella*. *International Journal for Parasitology* 29: 141-149.
- [5] Pozio E., La Rosa G., Serrano F.J., Barrat J., Rossi L. 1996. Environmental and human influence on the ecology of *Trichinella spiralis* and *Trichinella britovi* in Western Europe. *Parasitology* 113: 527-533.
- [6] Rossi L., Pozio E., Mignone W., Ercolini C., Dini V. 1992. Epidemiology of sylvatic trichinellosis in Northwestern Italy. *Revue des Sciences Techniques Office Internationale des Epizooties* 11: 1039-1046.
- [7] Pozio E. 1998. Trichinellosis in European Union: epidemiology, ecology and economic impact. *Parasitology Today* 14: 35-38.
- [8] Oivanen L., Oksanen A. 1994. Trichinellosis in domestic swine and wildlife in Finland. In: *Trichinellosis* (Eds. W.C. Campbell, E. Pozio, F. Bruschi). Istituto Superiore di Sanita Press, Rome, Italy: 569-574.
- [9] Cabaj W., Pozio E., Moskwa B., Malczewski A. 2000. *Trichinella britovi* and *T. spiralis* in red foxes (*Vulpes vulpes*) in Poland. *Acta Parasitologica* 45: 340-344.
- [10] Pavlíčková Z., Koudela B. 2004. The occurrence of animal trichinellosis in the Czech Republic. *Abstract book of Czech and Slovak helminthological days, May 17.-21., Ostravice, 2004*: 59.
- [11] Sréter T., Széll Z., Marucci G., Pozio E., Varga I.: 2003. Extraintestinal nematode infections of red foxes (*Vulpes vulpes*) in Hungary. *Veterinary Parasitology* 115: 329-334.
- [12] Akimov I.A., Didyk J.M., Schmalhausen, I.I. 2004. Trichinellosis of wild mammals in northwest Ukraine. *XIth International Conference on Trichinellosis, August 8-12, San Diego, California, 2004*: abstr. No. 67.
- [13] Oivanen L., Kapel C.M.O., Pozio E., La Rosa G., Mikkonen T., Sukura A. 2002. Associations between

- Trichinella* species and host species in Finland. *Journal of Parasitology* 88: 84-88.
- [14] Malakauskas A., Kapel C.M.O. 2002. Molecular epidemiology of *Trichinella* spp. in Lithuania. *Ph.D. Thesis*. Copenhagen, Denmark, 2002.
- [15] Cabaj W., Moskwa B., Pastusiak K., Bień J., Malczewski A. 2004. Trichinellosis in wild and domestic animals in Poland. *XIth International Conference on Trichinellosis, August 8-12, San Diego, California, 2004*: abstr. No. 68.

Wpłynęło 13 czerwca 2006  
Zaakceptowano 3 lipca 2006