



Trichinellosis in Slovakia – epidemiological situation in humans and animals (2009–2018)

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Abstract

Introduction. *Trichinella* spp. are zoonotic parasitic nematodes with almost worldwide distribution. The infection can be transmitted through the foodborne route and can cause serious health problems in infected human patients. It is also an economically important issue due to the high financial cost connected with prevention of the disease.

Objective. The study aimed to discover trends in the epidemiological situation in people and animals in Slovakia in 2009–2018.

Materials and method. Data on human trichinellosis originated from the Public Health Authority of the Slovak Republic, and data on *Trichinella* infection in animals from the State Veterinary and Food Administration of the Slovak Republic. A seroepidemiological study on 655 voluntary respondents was performed.

Results. Altogether, 29 human cases were reported between 2009–2018, with a maximum of 13 cases in 2011. Males were affected more often (19 cases) than females (10 cases); the average age of patients was 45.1 years. Antibodies to *Trichinella* were detected in two sera (0.3%): in a serum from one hunter and one veterinarian. In the monitored period, the average prevalence of *Trichinella* infection was 9.6% in 2,295 red foxes, and 0.04% in 165,643 wild boars. Three (1.7%) of 178 surveyed brown bears were positive. Within the compulsory monitoring of trichinellosis in domestic pigs, none from a total of 1,632,688 pigs were positive. The presence of three species, *Trichinella britovi*, *T. spiralis* and *T. pseudospiralis*, was documented, with *T. britovi* representing 93.9% of identified isolates.

Conclusions. The study indicates that the prevalence of *Trichinella* infection has not changed considerably with time in Slovakia, and the risk of human infection outbreaks is connected mainly with the consumption of wild boar meat.

Key words

Trichinella spp., Trichinellosis, human cases, free-living animals, pigs, Slovakia

INTRODUCTION

Trichinella spp. are parasitic nematodes with worldwide distribution on all continents, except Antarctica [1]. Infection can be transmitted through the foodborne route in carnivores and in omnivores with a scavenging behaviour [2], and can cause serious health problems in infected human patients. In addition, it is also an economically important issue due to the high financial cost connected with prevention of the disease at farm and slaughterhouse level, meat inspection and freezing of meat [3].

Currently, based on genetic data, 12 species and genotypes encompassed in two clades are recognized in the genus [4]. The encapsulated clade (infecting only mammals) includes six species and three genotypes (*T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, *T. nelsoni* and *T. patagoniensis*, and T6, T8 and T9 genotypes). The second, non-encapsulated clade, comprises three species (*T. pseudospiralis*, *T. papue* and *T. zimbabwensis*) that infect mammals, reptiles and birds [1].

Human trichinellosis has been documented in 55 (27.8%) countries around the world, with its occurrence strictly related to cultural food practices, including the consumption of raw or undercooked meat [5].

In Slovakia, *Trichinella* circulates primarily in the sylvatic cycle, with several sporadic human outbreaks registered since the 1930's. The presence of three species, *T. spiralis*, *T. britovi* and *T. pseudospiralis*, has been documented in the country [6, 7]. *T. spiralis* shows high infectivity and longest survival rate of larvae in domestic and wild pigs, compared to other *Trichinella* species. Thus, it is considered the main etiological agent of human trichinellosis [8]. *T. britovi* is considered less pathogenic, mainly due to lower reproductive capacity, and consequently lower numbers of newborn larvae in infected animals [7]. Non-encapsulated *T. pseudospiralis* is the only species infecting both mammals and birds. It has been documented mainly in the wildlife of almost all European countries [8, 9]. Two human outbreaks of *T. pseudospiralis* infection were reported in Europe, in 2000 in France and in 2015 in Italy [10, 11]. Despite preventive measures applied in Slovakia, new cases of human *Trichinella* infection are reported every year.

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OBJECTIVES

The aim of the study was to discover trends in the epidemiological situation in people and animals in last 10 years in Slovakia. A seroepidemiological survey was performed to obtain the data on the seropositivity to *Trichinella* in population with higher and lower risk of infection.

MATERIALS AND METHOD

Human trichinellosis – data collection. Data on human trichinellosis were collected in cooperation with the Public Health Authority of the Slovak Republic in the period of 2009–2018. According to the current legislation of the Slovak Republic, trichinellosis is classified into Group B of transmissible diseases. Group B comprises diseases that are compulsory reported to the Public Health Authority of the Slovak Republic. Occurrence or suspicion of the disease has to be reported within 24 hours. Data are processed in accordance with current legislation, namely by the Commission Implementing Decision C (2012) 5538 of 8 August 2012, amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No. 2119/98/EC of the European Parliament, and of the Council. Subsequently, data are processed and reported to the Epidemiological Information System (EPIS) in Slovakia and then to European Surveillance System (TESSy).

The criteria for establishing cases of human trichinellosis are based on clinical and laboratory findings, and epidemiological anamnesis. Clinical findings include: fever, muscle pain, diarrhoea, facial oedema, subconjunctival or retinal haemorrhages, haemorrhages of the nail beds, and eosinophilia. Laboratory criteria involve the specific antibody response to *Trichinella* detected by ELISA (Enzyme Linked Immunosorbent Assay), IFA (Immunofluorescence Assay), Western blot, or the presence of *Trichinella* larvae in the sample. Epidemiological anamnesis should include consumption of meat or meat products potentially positive to *Trichinella*. The case is established as probable if the patient suffers from clinical signs and the epidemiological anamnesis is positive. If clinical and laboratory criteria are met, the case is considered confirmed.

Seroepidemiological study in humans. Between 2015–2018, a total of 655 humans serum samples were collected from voluntary respondents who did not report clinical signs of acute disease at the time of blood collection. Personal and epidemiological data were gathered by a questionnaire which included informed consent with data processing for the seroepidemiological survey. Blood samples were taken under standard conditions using the Vacutainer System with the cooperation with clinicians.

Sampling focused on a population with a potentially higher risk of *Trichinella* infection (hunters; $n=98$), participants who could be in a contact with meat of infected animals (veterinarians; $n=80$) and 'other' participants ($n=477$). The studied group consisted of 403 men and 252 women; average age – 46.2 ± 20.8 years, ranging from 18–89 years.

Based on origin, 459 participants were from districts of eastern Slovakia, 35 from central Slovakia and 134 from western Slovakia.

Indirect ELISA was used for the detection of anti-*Trichinella* antibodies. *T. spiralis* larval somatic antigen was prepared according to Reiterová et al. [12]. Microtitre plates were coated with antigen containing 1.25 $\mu\text{g/ml}$ protein diluted in carbonate buffer (pH 9.6). Serum samples (diluted 1:100) were placed on plates in a volume of 100 μl per well. Anti-human IgG Immunoglobulin (Goat Anti-human IgG, Sigma-Aldrich, Missouri, USA) diluted 1:30,000 in a volume of 100 μl was used as a conjugate. Antibody reactions were visualized by adding 100 μl substrate (o-phenylenediamine/methanol diluted 1:100 with 0.05% H_2O_2). Sera of two patients with confirmed trichinellosis (obtained in the cooperation with Clinics of Infectology) and two negative sera were used as controls. Cut-off value was calculated according to optical density (OD) values of positive and negative controls, and samples with OD values higher than 40% of average OD of positive controls were considered positive.

ELISA-positive samples were also tested by the Western blot method according to Reiterová et al. [13], with some modifications. Excretory/secretory antigens of *T. spiralis* were separated on 12% SDS polyacrylamide gel under reducing conditions, and then transferred on nitrocellulose membrane in Tris-glycine buffer using Bio-Rad Trans-Blot Cell. After blotting, the membranes were cut into strips, blocked and incubated with serum samples and controls (diluted 1:50) for one hour at 37 °C. After washing, the strips were incubated with Goat Anti-Human IgG (Sigma-Aldrich, Missouri, USA) diluted 1:500 for an hour at 37 °C temperature. Afterwards, the bands were visualised using 4-chloro-1-naphtol and 0.03% hydrogen peroxide. The banding patterns were compared with positive and negative controls and molecular weight marker. Positive samples were characterized by specific bands localized between 42–44 kDa. Western blot was used as a confirmatory test for the ELISA-positive samples, and only samples positive by both tests were considered seropositive.

Animal *Trichinella* infection – data collection. Data about the occurrence of *Trichinella* spp. in red foxes (*Vulpes vulpes*), wild boars (*Sus scrofa*), and brown bears (*Ursus arctos*) between 2009–2018 were obtained with the cooperation of the State Veterinary and Food Administration of the Slovak Republic (SVFA SR). The prevalence data were collected within the regular monitoring of domestic swine, wild boars, horses and foxes, or other indicator animals for *Trichinella* infection in accordance with current legislation, namely by Commission Delegation (EC) No. 2075/2005 and by the Law about Veterinary Care No. 39/2007 [14], and by Commission Implementing Regulation (EU) 2015/1375.

According to the above-mentioned legislation, the Instructions for the monitoring of *Trichinella* infection are issued by SVFA SR every year. Approximately five red foxes, examined within the monitoring of oral antirabies vaccination, were sampled per each of 40 Regional Veterinary and Food Administrations. Data reported to the SVFA SR included the number of examined red foxes, the number of positive red foxes and, with the exception of 2009, as well as the origin of the animals. Wild boars hunted for own consumption (consumption of the hunter and his family) were examined on a voluntary basis, while animals intended for distribution were examined compulsorily. The data on wild boars reported to the SVFA SR included the number and origin of positive animals. Brown bears were examined

on a voluntary basis with their numbers and places of origin documented.

Domestic pigs from backyard slaughtering were monitored only from districts considered to be at a higher risk of trichinellosis (based on the positivity of wild boars and red foxes in the district). Pigs outside of 'risk districts' were examined on a voluntary basis. All pigs slaughtered at slaughter-houses and intended for human consumption were examined mandatory.

Samples were examined in the laboratories of the State Veterinary and Food Institutes or in the approved certified private laboratories. The magnetic stirrer method was used according to the Commission Implementing Regulation (EU) 2015/1375. In positive samples, the *Trichinella* species was identified by multiplex PCR recommended by the European Union Reference Laboratory for Parasites [15]. Pooled samples (at least four larvae) were tested from all *Trichinella* positive animals (n = 288).

Statistical analyses. Prevalence values of *Trichinella* infection in animals (red foxes and wild boars) and voluntary respondents were provided with a 95% confidence interval (95 % CI). Chi square (χ^2) test was used to test the differences among *Trichinella* prevalences in years and regions. Statistical analyses were performed by Quantitative Parasitology on the Web [16].

RESULTS

Trichinellosis in humans. Altogether, 29 human cases were reported to the Public Health Authority of the Slovak Republic between 2009–2018. The number of cases varied between 0–13 per year, with a minimum (no cases) in 2009 and 2018, and a maximum (13 cases) in 2011. Males were affected more often (19 cases) than females (10 cases). Average age of patients was 45.1 ± 17.3 years, and the majority of infected belonged to the age category of 45–54 years-old (7 persons) and 55–64 years-old (7 persons) (Tab. 1).

Infected patients came from six of eight regions of Slovakia. Two cases were recorded in Košice Region situated in eastern part of the country. Four patients came from central Slovakia (Žilina Region) and 23 patients from western territory of the country (Trenčín, Nitra, Trnava and Bratislava Regions) (Fig. 1).

Except one small family epidemic with three affected persons at the turn of 2011 and 2012, all reported cases were individual. In 2011, the disease was reported in a 76-year-old male from Tvrdosín district. Later, at the turn of 2011 and

Table 1. Human cases of *Trichinella* infection reported to Public Health Authority of the Slovak Republic, 2009–2018

Year	No. of cases	Gender	Age	District	Region
2009	0	–	–	–	–
2010	2	Female	35	Komárno	Nitra region
		Female	81	Michalovce	Košice region
		Male	39	Komárno	Nitra region
		Male	63	Komárno	Nitra region
		Female	63	Komárno	Nitra region
		Male	39	Komárno	Nitra region
		Female	54	Komárno	Nitra region
		Male	60	Komárno	Nitra region
2011	13	Female	48	Komárno	Nitra region
			52	Komárno	Nitra region
		Male	73	Levice	Nitra region
		Male	61	Prievidza	Trenčín region
		Female	41	Topoľčany	Nitra region
		Female	35	Námestovo	Žilina region
		Male*	76*	Tvrdošín*	Žilina region*
		Male*	48*	Tvrdošín*	Žilina region*
		Male*	47*	Tvrdošín*	Žilina region*
		2012	5	Male	47
Male	62			Komárno	Nitra region
Female	53			Komárno	Nitra region
Male	17			Bratislava	Bratislava region
Female	19			Hlohovec	Trnava region
2013	5			Male	31
		Male	21	Trnava	Trnava region
		Female	60	Trnava	Trnava region
2014	1	Female	56	Prievidza	Trenčín region
2015	1	Male	42	Komárno	Nitra region
2016	1	Male	66	Nitra	Nitra region
2017	1	Male	31	Trebišov	Košice region
2018	0	–	–	–	–

* family epidemic –

2012, clinical signs (muscle pain, swollen joints and sporadic abdominal pain) also appeared in his two 47 and 48 years-old sons, and the disease was reported to the Public Health Authority in 2012. All three males worked on a sheep farm and stated the consumption of meat and sausages from wild boars, deer and home-slaughtered pigs.

Epidemiological anamnesis was documented in seven other cases, five patients stated consumption of wild boar meat and two people consumed pork. Unfortunately, the causative agent of infection was not specified in any of 29 reported patients.

Seropositivity to *Trichinella* in voluntary respondents.

Within the epidemiological survey, 655 human serum samples were examined. ELISA positivity was recorded in three sera (0.5%), namely in one hunter, one veterinarian and one person classified as belonging to 'other participants'. Western Blot analysis confirmed the seropositivity in two samples (0.3%), in the serum of one hunter and one veterinarian (Tab. 2). The positive hunter was a 53-year-old

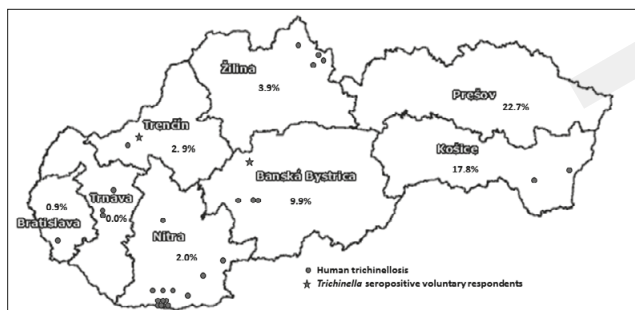


Figure 1. Occurrence of human cases of trichinellosis, voluntary respondents seropositive to *Trichinella* and the prevalence of *Trichinella* infection in red foxes in Slovakia, 2009–2018

Table 2. Seropositivity to *Trichinella* in participants of seroepidemiological survey

Group	Tested	ELISA positive (%) (95% CI)	WB positive (%) (95% CI)
Hunters	98	1 (1.0) (0.0 – 5.6)	1 / 1.0 (0.0 – 5.6)
Veterinarians	80	1 (1.3) (0.0 – 6.8)	1 / 1.3 (0.0 – 6.8)
Other participants	477	1 (0.2) (0.0 – 1.2)	0 (0.0) (0.0 – 0.6)
Males	403	2 (0.5) (0.1 – 1.8)	1 (0.2) (0.0 – 1.4)
Females	252	1 (0.4) (0.0 – 2.2)	1 (0.4) (0.0 – 2.2)
TOTAL	655	3 (0.5) (0.1 – 1.3)	2 (0.3) (0.0 – 1.1)

WB – Western blot; % – percentage; 95% CI – 95% Confidence Interval

male from the Trenčín region situated in western Slovakia, while the positive veterinarian was a 26-year-old female from Banská Bystrica region in central Slovakia (Fig. 1). They did not report any clinical signs of trichinellosis.

Trichinella infection in animals. Data on *Trichinella* infection in red foxes, wild boars and brown bears were reported to the State Veterinary and Food Administration of the Slovak Republic. Since 2009, 2,295 red foxes have been examined with a minimum of 181 animals in 2016 and a maximum of 433 foxes in 2012. The average prevalence rate was 9.6%, ranging from 2.8% in 2017 to 20.4% in 2011, with the differences among years being of statistical significance ($P < 0.001$) (Table 3).

Table 3. Prevalence of *Trichinella* spp. in domestic and free-living animals reported to State Veterinary and Food Administration of the Slovak Republic, 2009 – 2018

Year	Animal species							
	Red foxes		Wild boars		Brown bears		Pigs	
	N / n	% (95% CI)	N / n	% (95% CI)	N / n	% (95% CI)	N / n	%
2009	13/193	6.7 (3.6 – 11.3)	4/12,605	0.03 (0.0 – 0.1)	0/25	0.0 (0.0 – 11.3)	0/153,585	0.0
2010	21/211	9.9 (6.3 – 14.8)	11/26,895	0.04 (0.0 – 0.1)	1/37	2.7 (0.0 – 14.2)	0/147,328	0.0
2011	38/186	20.4 (14.9 – 26.9)	4/15,405	0.03 (0.0 – 0.1)	0/0	–	0/140,907	0.0
2012	43/433	9.9 (7.3 – 13.1)	10/14,377	0.07 (0.0 – 0.1)	0/26	0.0 (0.0 – 10.9)	0/121,370	0.0
2013	38/358	10.6 (7.6 – 14.3)	10/13,737	0.07 (0.0 – 0.1)	1/17	5.9 (0.2 – 28.7)	0/628,006	0.0
2014	16/209	7.7 (4.4 – 12.1)	6/15,575	0.04 (0.0 – 0.1)	1/8	12.5 (0.3 – 52.7)	0/95,398	0.0
2015	17/201	8.4 (5.0 – 13.2)	7/16,342	0.04 (0.0 – 0.1)	0/21	0.0 (0.0 – 13.3)	0/94,231	0.0
2016	19/181	10.5 (6.4 – 15.9)	7/14,424	0.05 (0.0 – 0.1)	0/10	0.0 (0.0 – 25.9)	0/81,931	0.0
2017	6/211	2.8 (1.1 – 6.1)	3/12,575	0.02 (0.0 – 0.1)	0/16	0.0 (0.0 – 17.1)	0/88,221	0.0
2018	9/112	8.0 (3.7 – 14.7)	3/13,708	0.02 (0.0 – 0.1)	0/18	0.0 (0.0 – 15.3)	0/81,711	0.0
TOTAL	220/2,295	9.6 (8.4 – 10.9)	65/155,643	0.04 (0.0 – 0.1)	3/178	1.7 (0.4 – 4.9)	0/1,632,688	0.0

N – number of positive; n – number of tested; % – percentage; 95% CI – 95% Confidence Interval

Table 4. *Trichinella* species detected in free-living animals in Slovakia, 2009 – 2018

Species	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total (%)
<i>Trichinella britovi</i>	17	17	28	42	40	17	14	6	8	11	200 (69.4)
<i>Trichinella spiralis</i>	-	-	-	-	3	1	-	-	-	-	4 (1.4)
<i>Trichinella pseudospiralis</i>	-	-	-	-	-	-	-	-	1	-	1 (0.3)
<i>Trichinella</i> spp.	-	16	14	11	6	5	10	20	-	1	83 (28.8)

Although the number of examined wild boars was much higher (165,643), the positivity was lower in this host and reached 0.04%, on average, ranging between 0.02% in 2017 and 2018 to 0.07% in 2012 and 2013, but the observed differences were not statistically significant ($P > 0.05$).

The average prevalence in brown bears was 1.7%. Three bears were positive among 178 surveyed animals. Within the compulsory monitoring of *Trichinella* infection in domestic pigs, altogether 1,632,688 pigs were examined, but the positivity was not detected in any animal (Tab. 3).

Geographically, the prevalence of infection in red foxes in the period of 2010–2018 varied significantly among regions ($P < 0.001$). The highest number of positive animals came from eastern Slovakia, with 22.7% positivity in Prešov and 17.8% positivity in Košice Region. In central regions, the prevalence was lower, and in the area of western Slovakia ranged between 0.0–2.9% (Fig. 1). Unfortunately, the exact numbers of wild boars examined per each region were not reported to the SVFA SR. Thus, only the occurrence of positive wild boars could be evaluated, and statistical analyses of inter-regional differences could not be performed. Of a total of 65 positive animals, 38.5% (25) wild boars were hunted in eastern Slovakia (Košice and Prešov Regions), 47.7% (31) animals were hunted in central parts of the country (Žilina and Banská Bystrica Regions), and 4.6% (3) of positive wild boars came from regions situated in western Slovakia (Trenčín, Trnava, Nitra and Bratislava Regions). The origin of four animals was not recorded.

All three positive brown bears came from central Slovakia (Žilina Region); two bears reported in 2010 and 2014 came from areas situated only approximately 10 km apart (Liptovský Hrádok and Pribylina), while the animal hunted in 2013 was from the area (Kraľovany) located approximately

60 km from Liptovský Hrádok and 55 km from Pribylina.

Molecular identification of *Trichinella* isolates detected in free-living animals (n=288) between 2009–2018 revealed that *Trichinella britovi* was the predominant species (69.4%), followed by *T. spiralis* (1.4%) and *T. pseudospiralis* (0.3%). Mixed infection was not recorded in any animal and the species of *Trichinella* was not identified in 28.8% isolates (Tab. 4). When only recognized *Trichinella* species are taken into consideration, *T. britovi* presented 93.9% of isolates, 5.5% isolates were identified as *T. spiralis*, and 0.6% isolates as *T. pseudospiralis*. *Trichinella spiralis* was detected in four red foxes originating from eastern Slovakia (Prešov district). Three animals were positive in 2013 and one red fox in 2014. *Trichinella pseudospiralis* was recorded in one animal, a wild boar hunted in central Slovakia (Žilina Region) in 2017.

DISCUSSION

The study confirmed constant circulation of *Trichinella* spp. in wildlife of Slovakia and the almost annual occurrence of new human cases. The occurrence of individual cases prevailed over the epidemics, as only one small family epidemic with three infected males was reported between 2009–2018, while the remaining 26 cases were individual.

Based on the results, human trichinellosis occurs throughout the country, as infected patients came from six of eight of Slovak regions, situated in eastern (Košice Region), central (Žilina Region) and western (Trenčín, Nitra, Trnava and Bratislava Regions) parts of the country. In the past, only eastern Slovakia had been considered an area where human trichinellosis occurred. Between 1939–1980, twelve human epidemics occurred, all of them recorded in eastern Slovakia [17]. Later, in 1998, human trichinellosis was recorded in central (Brezno district) and in 2008, for the first time also in the western part (Komárno district) of the country [7]. In the presented study, the majority (23) of reported cases were recorded in western districts.

Human trichinellosis occurs also in the countries neighbouring to Slovakia. In Poland, bordering with Slovakia to the north, trichinellosis is considered an ongoing epidemiological problem with a total of 935 cases reported between 2000–2013, ranging from one to 292 cases per year [18]. In contrast, human trichinellosis in the Czech Republic and Hungary seems not to be of great epidemiological importance. Altogether, 31 cases were recorded in the Czech Republic between 1986–2009 [19]. Since 2010, five new cases have been reported to the National Institute of Public Health of the Czech Republic [20]. In Hungary, 573 cases of imported or autochthonous *Trichinella* infection were documented between 1965–2009 [21]. Later, a small outbreak of the disease in eight persons occurred after the consumption of pork and smoked sausages from *T. spiralis* infected pig [22], but no real epidemiological data have been published.

A seroepidemiological study aimed at estimating the risk of *Trichinella* infection in the population of Slovakia revealed only two (0.31%) seropositive persons of 655 examined: a hunter from western Slovakia and a veterinarian from the central part of the country. The occurrence of trichinellosis in people is strictly related to cultural food practices, mainly the consumption of raw or undercooked meat [5]. Due to direct contact with meat of hunted game and its further processing and consumption, hunters are

considered to be at higher risk of *Trichinella* infection. In Poland, 17 (1.7%) of 1,027 hunters were seropositive in a study by Sadkowska-Todys et al. [23]. Hunting activities were also significantly associated with *Trichinella* seropositivity in East Greenland, where altogether 3.1% of 998 examined people were seropositive [24].

Persons seropositive in the presented study did not report clinical signs of the disease. In general, the course and severity of human trichinellosis depends on the *Trichinella* species involved, the infective dose and the phase of the infection. Often, the infection is asymptomatic or connected only with mild symptoms [2, 25]. Thus, the presence of antibodies in positive persons can signalise asymptomatic or former infection caused by low number of *Trichinella* spp. larvae.

The study showed that the main reservoir animal of *Trichinella* spp. in Slovakia is the red fox, in which the average prevalence of *Trichinella* infection was 9.6%, ranging between 2.8% in 2017 and 20.4% in 2011, with statistically significant differences. Nevertheless, no time dependency, it means continual decrease or increase of the prevalence, was recorded in the monitored period. Slightly higher, 11.5% average prevalence of infection, was recorded in the long term-survey conducted in the country between 2000–2007, with totally 5,270 red foxes examined [6]. At that time, the prevalence rate ranged between 4.9% in 2000 and 20.5% in 2007.

The positivity of wild boars has also showed the stable levels, with an average prevalence of 0.04% recorded in the presented study, and 0.06% prevalence detected in the study by Hurníková and Dubinský [6]. Thus it seems that despite of significant changes of prevalence of infection in red foxes between the surveyed years, the mean prevalence in both animal species has not considerably changed with time in Slovakia. Similarly, no relation seems to exist between the continual growth in the population of red foxes and wild boars in Slovakia and the incidence of *Trichinella* infection. The number of red foxes has continually risen from 26,063 in 2009 to 32,895 in 2018; and the number of wild boars has increased from 31,652 in 2009 to 41,723 in 2018 [26, 27]. Nevertheless, the observed changes in the prevalence of infection did not correlate with the documented growth of animal populations.

The trend of geographical distribution or spread of *Trichinella* infection seems to have similar features in both, people and animals. The first signs of the spread of animal *Trichinella* infection towards the west were recorded by Hurníková and Dubinský [6]. They found no positive red fox in central and western regions in 2000 and 2001, the first sporadic findings occurred in 2003 and 2004, and more than 15% positivity in 2006 and 2007. The results obtained in the current study showed the positivity of red foxes and wild boars in the whole country, but the highest prevalence of infection in red foxes still persists in eastern Slovakia (17.8% positivity in Košice Region and 22.7% in Prešov Region) and the lowest in western areas (0.0% in Trnava and 0.9% in Bratislava Region). In humans, trichinellosis in the past was recorded only in eastern Slovakia, but the presented study has confirmed its common occurrence also in western parts of the country. Nevertheless, due to frequent travelling and tourist activities and the transport of meat and meat products within and between countries, the occurrence of the disease in humans cannot be used to evaluate the real epidemiological situation.

As in the past, *Trichinella britovi* has remained the predominant species circulating in Slovakia, while *T. spiralis* and *T. pseudospiralis* occurred only sporadically. *T. pseudospiralis* was detected in 2003 for the first time in Slovakia in domestic pigs, cat and rats at a pig breeding farm in eastern Slovakia. In 2005 and 2006, the parasite was found in wild boars and red foxes (co-infection with *T. britovi*), and later also in birds of prey. All positive animals and birds originated from eastern Slovakia [6, 28, 29]. The finding here of reported wild boar hunted in central Slovakia confirms the spread of *T. pseudospiralis* through the territory of the country via birds or other free-living hosts.

The situation in southern situated Hungary, with 2.1% *Trichinella* positive red foxes and 0.02% positive wild boars, and the occurrence of three species, predominant *T. britovi*, followed by *T. spiralis* and less frequent *T. pseudospiralis*, is similar to that in Slovakia [30]. Towards the west, only sporadic occurrences of *Trichinella* infection in animals is reported from the Czech Republic. In 2016, three wild boars were positive of 164,000 tested. In 2018, 68,300 animals were tested and only one was confirmed positive, and similarly, only one wild boar was positive among 71,000 examined by August 2019 [31]. On the contrary, in Poland (to the north of Slovakia) the animal *Trichinella* infection is more prevalent and has been detected not only in red foxes and wild boars, but also in pigs. During the 10-year study period (2006–2015), infection was detected in 282 pigs and in 0.51% of 1,012,021 wild boars [32]. In this country, the presence of all three species occurring also in Slovakia has been recorded. Between 2009–2012, only *T. spiralis* and *T. britovi* were reported in wild boars within the compulsory monitoring performed in Poland by the Veterinary Inspection Service [33]. In 2012 the first two cases of *T. pseudospiralis* were documented. The parasite was detected in a wild boar from West Pomeranian Province situated in the north-west of Poland [34], and in a red fox hunted in the south of the country, in the area bordering Slovakia [35], only 100 km air distance from the locality where *T. pseudospiralis* positive wild boar was hunted in Slovakia. While in Poland *T. spiralis* predominates over *T. britovi* and both species occur throughout the country [33]; in Slovakia the occurrence of *T. spiralis* is less frequent and limited only to its eastern areas.

The study indicates that the prevalence of *Trichinella* infection has not considerably changed with time in Slovakia. Presented data showed its constant presence in the country with the slow spread towards the west, noticeable in both animals and humans. With the exception of wild boars and red foxes, *Trichinella* infection in Slovakia was also identified in other wild animals (martens, European polecat and brown bear) and birds of prey, which can thus represent other parasite reservoirs [6, 29]. Nevertheless, regarding the population density of wild boars and red foxes that have been considered overpopulated in Slovakia for several years, these two species are of major importance for the spread of *Trichinella* infection in the country. On the other hand, due to the high population density of wild boars, the alimentary mode of infection and quite common use of wild boar meat for cooking (especially in hunters and their families), wild boar meat constitutes a major threat to people in Slovakia. However, it is worth mentioning that an indirect threat also exists because of the possibility of *Trichinella* transmission through the domestic cycle.

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Conflict of interest

None.

Ethical standards

The study was performed in accordance with the 1975 Declaration of Helsinki, as revised in 2013, and was approved by the Ethics Committee of Institute of Parasitology of SAS (No. EK 04/2015). All respondents or their legal representatives agreed with the survey and signed the informed consent. No identifying data are presented in the paper.

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