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The role of wild boars (*Sus scrofa)* in circulation of trichinellosis, toxocarosis and ascariosis in the Slovak Republic

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Summary

Wild boars (*Sus scrofa*) can play a significant role in circulation and maintenance of certain parasites in the environment. The aim of this study was to determine the seroprevalence of trichinellosis, toxocarosis and ascariosis in wild boars hunted in the Slovak Republic in 2003 and 2004. Anti-*Trichinella* antibodies were detected in 1.3 % out of 1035 wild boars investigated. No significant differences were observed regarding the positivity of wild boars according to age and sex in both years. The examination of 1173 wild boars for anti-*Toxocara* antibodies revealed 7.2 % seropositive individuals. Anti-*Ascaris* antibodies were detected in 6.1 % out of 411 animals examined. Both, anti-*Trichinella* and anti-*Toxocara* antibodies were determined in 0.6 % cases. Presence of anti-*Trichinella* and anti-*Ascaris* antibodies was recorded in 0.8 % animals. Concurrent infection caused with *Toxocara* and *Ascaris* was observed in 1.5 % individuals. This study concentrates on the role of wild boars in circulation and on the maintenance of trichinellosis, toxocarosis and ascariosis in the Slovak Republic.

Key words: wild boars; *Trichinella* spp.; *Toxocara* spp; *Ascaris suum*; seropositivity

Introduction

The wild boar (*Sus scrofa*) is a very important game species in Slovakia whose number has significantly increased during the last decade. According to the Slovak Hunting Association (2004) the number of wild boars was 17,738 in the spring of 1995, while in 2002 it increased to 26,135 animals. Because of their feeding habits, especially necrophagy, they can be responsible for the spread of trichinellosis, a serious helmithozoonosis acquired by consumption of raw or insufficiently cooked meat containing *Trichinella* spp. larvae. In Slovakia wild boar meat is the most common source of human trichinellosis (Dubinský, 1997).
Wild boars can also be infected with *Toxocara* spp., and thus serve as paratenic hosts and contribute to its persistence in the environment. After the ingestion of infective *Toxocara* spp. eggs, larvae hatch in the small intestine, migrate via blood vessels and then remain as somatic larvae in their tissues for many years. Infection can also arise after the ingestion of somatic larvae present in tissues of other paratenic hosts (Overgaauw & Van Knapen, 2000). In humans, infected with *Toxocara* spp. eggs or larvae, this migration causes the complex of symptoms called *larva migrans visceralis* (VLM) or *larva migrans ocularis* (OLM). Milder form of human toxocarosis, called covert toxocarosis, is also very common.
Ascaris suum, a large roundworm of pigs, is found commonly in wild boars (De-la-Muela *et al*., 2001; Fernandez-de-Mera *et al*., 2003). Human consumption of infective *A. suum* eggs can lead to migration of hatched larvae through portal and lymphatic circulatory system to liver and lungs or other organs, causing *larva migrans visceralis* (Dubinský, 1993).
The aim of this study was to determine the seroprevalence of chosen helminthozoonoses caused by *Trichinella*, *Toxocara* and *Ascaris*, in wild boars hunted in the Slovak Republic in 2003 and 2004.

Material and Methods

Wild boar sera
Wild boars hunted in different regions of the Slovak Republic throughout the period of May – July 2003 and in October 2004 within the monitoring of classical swine fever were included in this study. Immediately after the death, blood and muscle samples were collected. After centrifugation at 3000 rpm for 10 min, sera were collected and stored at – 20 °C until tested. Muscle samples were examined for *Trichinella* larvae by the digestion method (The magnetic stirrer method for pooled samples) at the State Veterinary Institute, Zvolen. The sera were sent for serological examination to the Parasitological Institute SAS, Košice. In both years, data about the age and in 2004

also about the sex were collected. According to age, wild boars were divided into 3 groups: under 1 year, between 1 and 2 years and animals over 2 years of age were classified as adults.
In 2003, a total of 636 wild boar sera was investigated for the presence of anti-*Trichinella* antibodies and 762 samples for the presence of anti-*Toxocara* antibodies. In 2004, a total of 399 sera were examined for *Trichinella* antibodies, 411 serum samples for *Toxocara* antibodies and 411 samples for *Ascaris* antibodies.

Detection of anti-Trichinella antibodies
Trichinella spiralis excretory-secretory (E/S) antigen was prepared according to Reiterová *et al*. (1999). For the detection of anti-*Trichinella* antibodies, microtitre plates were coated with 100 µl per well of E/S antigen *T. spiralis* containing 1.25 µg/ml protein diluted in phosphate buffer saline (PBS), pH 7.2, overnight at 4°C. Then they were washed three times with PBS containing Tween 20 (PBS-T). The plates were blocked with 100 µl per well of 2 % skimmed milk in PBS-T for 1 hour at a room temperature (21 ± 3°C). Serum samples (diluted in a proportion of 1:20 in 5 % bovine serum albumin in PBS-T) were placed onto plates in a volume of 100 µl per well. Sera of pigs experimentally infected with *T. spiralis* were used as positive controls and sera of uninfected pigs, confirmed by the digestion method to be negative, were used as negative controls. After 30 minutes incubation at a room temperature the plates were repeatedly washed 3 times. Protein G, recombinant-peroxidase labelled conjugate (Sigma), prediluted in a proportion of 1:320 in preservative solution, was diluted in a proportion of 1:100 in PBS-T with 0.25 % bovine serum albumin and added in a volume of 100 µl per well. After a 30-minute incubation at a room temperature the plates were washed 3 times and 100 µl of substrate (3, 3′, 5, 5′-Tetramethylbenzidine Liquid Substrate System, Sigma) was added. The reaction was stopped after about 20 min of incubation in the dark at the room temperature by 100 µl of 2 M H_2SO_4 and the optical density was measured spectrophotometrically at 450 nm (Thermo Labsystems Opsys MR, USA). For each sample, S/P % was calculated according to scheme:
S/P % = (OD value of the sample) / (OD value of positive control) x 100
As the lowest S/P % value of wild boar serum which, corresponded with a positive result by digestion method was 75 %, we considered samples with S/P % ≥ 75 % as positive.

Detection of anti-Toxocara and anti-Ascaris antibodies
Toxocara canis larval excretory-secretory antigen and *Ascaris suum* larval E/S antigen were prepared by the modification of the method of Havasiová-Reiterová *et al.* (1995). For determination of anti-*Toxocara* antibodies, the plates were coated overnight at 4°C with 100 µl / well of E/S *T. canis* antigen containing 1 µg/ml protein diluted in carbonate buffer (pH 9.6). Similarly, E/S *A. suum* was used under the same conditions as mentioned above. The follow-up process was the same in both, anti-*Toxocara* and anti-*Ascaris* antibody detection, respectively. The plates were washed three times with PBS-T and than blocked with 100 µl per well of 2 % skimmed milk in PBS-T for 1 hour at a room temperature (21 ± 3°C). Serum samples (diluted in a proportion of 1:100 in 5 % skimmed milk in PBS-T) were placed to plates in a volume of 100 µl per well. After one-hour incubation at 37°C, the plates were repeatedly washed 3 times. The rest of the investigation was the same as in the case of anti-*Trichinella* antibody detection. Since we have no positive and negative control sera for detection of toxocarosis and ascariosis, the cut-off was determined according to Naguleswaran *et al*. (2004): the first cut-off value was determined by the mean of all sera + 3 standard deviations (SD). Sera with optical density (OD) above this value were excluded and the remaining sera were used for calculation of mean absorption of negative samples ($M_{neg.}$) and $SD_{neg.}$. Sera with OD values above $M_{neg.} + 4\ SD_{neg.}$ were considered to be positive.

Data analysis
The significance of the differences in seropositivity of the mentioned parasitoses according to the age and sex of animals was evaluated by the chi-square test. Fisher exact test was used to establish seropositivity differences between year 2003 and 2004.

Results

Seropositivity to Trichinella spp.
Although *Trichinella* larvae were found by the digestion method only in 3 (0.47 %) out of 636 wild boars investi-

Table 1. Seroprevalence of *Trichinella* spp., *Toxocara* spp. and *Ascaris suum* in wild boars in 2003 and 2004 according to their age

Age category	*Trichinella* spp.			*Toxocara* spp.			*Ascaris suum*
	Year 2003 (N/n/%)	Year 2004 (N/n/%)	Total (N/n/%)	Year 2003 (N/n/%)	Year 2004 (N/n/%)	Total (N/n/%)	Year 2004 (N/n/%)
< 1 year	2/66/3.0	1/199/0.5	3/265/1.1	2/70/2.9	12/204/5.9	14/274/5.1	13/204/6.4
1 – 2 years	6/541/1.1	2/138/1.4	8/679/1.2	60/670/9.0	7/144/4.9	67/814/8.2	7/144/4.9
Adults	1/20/5.0	1/56/1.8	2/76/2.6	2/14/14.3	2/57/3.5	4/71/5.6	5/57/8.8
Unknown	0/9/0.0	0/6/0.0	0/15/0.0	0/8/0.0	0/6/0.0	0/14/0.0	0/6/0.0
Total	9/636/1.4	4/399/1.0	13/1035/1.3	64/762/8.4	21/411/5.1	85/1173/7.2	25/411/6.1

N – number of positive samples; n – number of examined samples; % – seropositivity

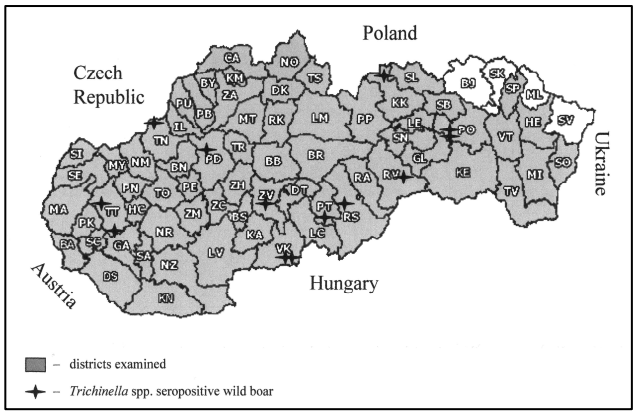

Fig. 1. Occurrence of *Trichinella* spp. seropositive wild boars in the Slovak Republic

gated in 2003, anti-*Trichinella* antibodies were detected in 9 (1.4 %) animals. When comparing the occurrence of seropositivity according to age, a prevalence of 5.0 % was recorded in adults, in wild boars between 1 and 2 years of age a 1.1 % positivity was observed and 3.0 % of animals younger than 1 year were positive (Table 1) with differences between age groups being not significant (p = 0.34; X = 3.39; df = 3). In 2004, no *Trichinella* larvae were detected by digestion of 399 muscle samples, but 4 (1.0 %) serum samples investigated serologically were positive. The difference in the infection rate between 2003 and 2004 was not statistically significant (p = 0.78). Wild boars originated from 67 districts of the Slovak Republic and seropositive animals were recorded in 11 districts (Fig. 1). In 2004, the higher prevalence was recorded in adult wild boars (1.8 %) rather than in animals under 1 and between 1 and 2 years of age (0.5 % and 1.4 %, respectively) (Table 1). The males were more frequently positive than females (1.4 % and 0.6 %, respectively) (Table 2), but no significant associations were observed between the age categories (p = 0.76, X = 1.16, df = 3), and sex (p = 0.47; X = 0.87; df = 2).

Seropositivity to Toxocara spp.

In 2003, an examination of 762 wild boars revealed 64 seropositive individuals, representing a prevalence of 8.4 %, while in 2004, out of a group of 411 investigated animals there were 21 (5.1 %) carrying anti-*Toxocara* antibodies (Table 1). This difference is not significant statistically (p = 0.058). Meanwhile, in 2003 the prevalence of *Toxocara* antibodies in adults was 14.3 % in comparison with wild boars between 1 and 2 years and younger than 1 year with 9.0 % and 2.9 %, respectively. In 2004 the animals up to 1 year yielded 5.9 % and wild boars between 1 and 2 years and adults showed seropositive results in 4.9 % and 3.5 %, respectively (Table 1). No significant differences in seroprevalence were observed concerning age (p = 0.27; X = 3.89; df = 3 and p = 0.84; X = 2.71; df = 3, respectively). Similarly, there was no significant difference (p = 0.68; X = 0.77; df = 2) between the seropositivity of males and females (5.6 % and 4.9 %, respectively) in 2004 (Table 2). Totally 869 animals were investigated for the presence of both, anti-*Trichinella* and anti-*Toxocara* antibodies in 2003 and 2004. A mixed infection was determined in 5 (0.6 %) wild boars (Table 3).

Seropositivity to Ascaris spp.

In 2004, out of 411 wild boars examined, anti-*Ascaris* antibodies were detected in 25 of them (6.1 %). The highest seropositivity was detected in adults (8.8 %) followed by animals under 1 year (6.4 %) and those between 1 and 2 years of age (4.9 %) (Table 1). With regard to the sex, the occurrence of antibodies in females was more frequent (7.7 %) than in males (5.1 %) (Table 2). No significant association was observed between the infection rate of age categories (p 0.72; X = 1.35; df = 3) and both sexes (p = 0.41, X = 1.79, df = 2).

Out of 399 wild boars investigated for the presence of both, anti-*Trichinella* and anti-*Ascaris* antibodies, mixed infection was recorded in 3 (0.8 %) animals and in 6 (1.5 %) individuals out of 411 examined, both anti-*Toxocara* and anti-*Ascaris* antibodies were observed (Table 3).

Table 2. Seroprevalence of *Trichinella* spp., *Toxocara* spp. and *Ascaris suum* in wild boars in 2004 according to their sex

Helminths	Male		Female		Unknown		Total	
	N/n	%	N/n	%	N/n	%	N/n	%
Trichinella spp.	3/208	1.4	1/179	0.6	0/12	0.0	4/399	1.0
Toxocara spp.	12/215	5.6	9/183	4.9	0/13	0.0	21/411	5.1
Ascaris suum	11/215	5.1	14/183	7.7	0/13	0.0	25/411	6.1

N – number of positive samples; n – number of examined samples; % – seropositivity

Table 3. Occurrence of mixed infection in wild boars

Parasites	N/n	%
Trichinella spp./*Toxocara* spp.	5/869	0.6
Trichinella spp./*Ascaris suum*	3/399	0.8
Toxocara spp./*Ascaris suum*	6/411	1.5

N – number of samples positive for both parasites; n – number of examined samples; % – seropositivity

Discussion

Circulation of helminthes in biocenosis is provided by biological peculiarities of helminthes themselves, their intermediate and final hosts, as well as a number of other com-

ponents of biocenosis, which are not directly involved in the parasitic life cycles (Anisimova, 2004).

In recent years, despite the intensive hunting of wild boars, their population has markedly increased in many European countries. The extensive cultivation of corn, which serves as an optimal food source in fields and also winter-feeding of game create very favourable living conditions for wild boars (Mercel, 2004). Sufficiency of food sources together with deficient hunting lead to the growth of their numbers, which eventually plays an important role in the spread of *Trichinella* spp. in the sylvatic cycle (Nöckler, 2003). In Slovakia, it is the red fox (*Vulpes vulpes*) that represents the main reservoir of trichinellosis. During an extensive survey in years 2000 – 2003, Dubinský *et al.* (2004) examined by digestion method 2518 red foxes. During this period, the 4.9 % prevalence recorded in 2000 increased to 16.8 % in 2003. Small mammals can serve as source of trichinellosis for both, red foxes and wild boars. In the recent study we observed 6.2 % seroprevalence of anti-*Trichinella* antibodies in 1125 small mammals examined. Animals investigated were divided into 15 species belonging to the order Rodentia and Insectivora (unpublished data). Ingestion of infected small mammals or carcasses of red foxes can lead to a wild boar infection. Besides the regions with endemic occurrence of *Trichinella* (Eastern and Central Slovakia), seropositive animals were detected also in the districts of Western Slovakia (TN – Trenčín, TT – Trnava, PD – Prievidza). Although, no wild boar was positive by digestion method in Western Slovakia to date, a spread of *Trichinella* towards the West cannot be excluded. According to Hurníková *et al*. (2004) *Trichinella* spp. larvae were detected only in endemic areas of East and Middle Slovakia in red fox population in 2000 but in 2001 and 2002 infected animals were recorded also in the districts in Western Slovakia. Similarly, Pavlíčková & Koudela (2004) found *Trichinella* in red foxes and wild boars in border regions of the Czech and Slovak Republic. Our results confirm the importance of wild boars in maintenance of the sylvatic *Trichinella* cycle. Up to now, in the Slovak Republic mainly *Trichinella britovi* which is moderately infective but persistent also in pigs and wild boars (Kapel & Gamble, 2000), was detected in wild boars (Hurníková *et al*., 2005). Despite the low number of animals resulting positive by the digestion method, the number of serologically positive animals was higher. Several authors obtained similar results, for example, in Belgium Geerts *et al.* (1995) found that none of 88 muscle samples digested were positive, but 8 serum samples reacted positively in ELISA. In Switzerland, Gottstein *et al*. (1997) detected anti-*Trichinella* antibodies in 31 (8.7 %) of 325 investigated wild boars and found no larvae with subsequent digestion method; and Van der Giessen *et al.* (2004) observed a 7.4 % seroprevalence in 107 wild boars tested in the Netherlands but only 1 animal was positive by digestion method. This discrepancy can be due to higher sensitivity of ELISA (0.01 larvae per gram – LPG) than that of the digestion method (± 1 LPG) (Madden & Murrell, 1990), what means that infections with a low number of muscle larvae would be misdiagnosed by digestion method. Kapel & Gamble (2000) observed in pigs inoculated with *T. britovi* the reduction of LPG by approximately 60 % from week 5 to week 40. In wild animals, the intensity of larval burden after infection may depend on the persistence of antibodies from preceding exposure (Martínez *et al*., 2004). We suppose that at re-infection the muscle larvae die and disappear due to antibody response. In our study, the highest seropositivity was detected in adult wild boars in both monitored years. Younger animals were less positive, probably due to their shorter contact with infected animals or their carcasses.

Toxocarosis is another helmithozoonosis that can be transmitted to humans by consumption of insufficiently cooked meat of some paratenic hosts. Taira *et al*. (2004) confirmed the zoonotic risk after consumption of *T. canis* infected pig viscera and thus we assumed a similar problem connected with consumption of raw or insufficiently cooked wild boar meat. The difference of *Toxocara* seropositivity detected in 2003 (8.4 %) and in 2004 (5.1 %) was not significant and there was no association between the age and occurrence of anti-*Toxocara* antibodies in monitored years. This could be caused by a different degree of environmental contamination with *Toxocara* spp. eggs or larvae in reservoir hosts. Red foxes are considered to be the main source of *Toxocara* eggs in the countryside, for example Antolová *et al*. (2004) recorded 8.1 % occurrence of *Toxocara* spp. eggs in 310 red foxes investigated coprologically. The authors also examined 710 small mammals and anti-*Toxocara* antibodies were detected in 7.7 % of the animals. Therefore, we suppose that a high prevalence of *Toxocara* in natural or paratenic hosts can lead to a higher prevalence in wild boars. This is the first time, when the occurrence of mixed infections in a large number of wild boars was monitored serologically. We observed concurrent seropositivity for *Toxocara* and *Trichinella* in 5 wild boars out of 869 examined.

Ascaris suum is often found as a parasite in wild boars. Human ingestion of infective *A. suum* eggs can cause *larva migrans visceralis* and occasionally adult worms are found in the intestine of man. In the life cycle of *A. suum* also paratenic hosts (earthworms, dung beetles and some insect) play a role and thus ascariosis, similarly to toxocarosis, can be present in the environment for a long time. Despite a higher susceptibility of younger animals (Dubinský, 1993) the highest positivity for anti-*Ascaris* antibodies was detected in adult animals. Because of the immunity, which develops after the first infection with *A. suum* and protects against the re-infection (Dubinský, 1993), we suppose that a long-term contact with the contaminated ecosystem is the main factor which may influence the prevalence of *Ascaris*. Nevertheless, some authors describe cross-reactions occurring in serodiagnosis of *A. suum* and *T. canis* infections (Kennedy *et al*., 1989), other confirmed the specificity of E/S *T. canis* and E/S *A. suum* antigens, with no cross-reactions observed in mice infected with *A. suum* eggs (Cuéllar *et al*., 1995) or in mice infected with *T. canis* eggs (Fan & Su, 2004). It was also confirmed by our re-

sults, that mixed infection with both, *Toxocara* and *Ascaris* spp. (1.5 %) may occur in wild boars. Simultaneous infection of *Trichinella* and *Ascaris* spp., was also determined in 3 out of 399 wild boars.

In conclusion, our study confirmed the important role of wild boars in circulation and maintenance of *Trichinella*, *Toxocara* and *Ascaris* spp. in the Slovak Republic and their zoonotic potential for human diseases, especially in hunters and in people who manipulate or consume wild boar meat-products.

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