PREVALENCE AND INTENSITY OF SARCOSPORIDIA INFECTION IN WILD CLOVEN-HOOFED HUNTED FOR FOOD IN LITHUANIA

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Abstract. Recent investigations have shown that the intensity of Sarcocystis infection in game animals used for food is high and in some countries even exceeds 90%. Sarcosporidia found in the muscle tissue of domestic and wild animals pose a potential risk to humans. Therefore, their investigations are rather important. Infection with Sarcosporidia (*Sarcocystis* spp.) cysts was evaluated in the main species of wild animals used for food: elks (n = 27), red deer (n = 54), sika deer (n = 45), roe (n = 36), and wild boars (n = 127). Sarcocystis cysts were found in all examined groups of muscles: esophagus, diaphragm, heart, neck, mandible, dorsum and leg. Depending on the animal species and examined groups of muscles, the prevalence of infection, determined microscopically in 1,279 muscle samples by compression slide method using methylene blue as an indicator dye, ranged from 46.43% to 100.0%.

Keywords: meat, game animal, Sarcocystis, prevalence and intensity of infection

Introduction. It is assumed that over 200 zoonoses including sarcocystosis can be transmitted from animals to humans (Frenkel, Smith, 2003). Herbivores and frugivorous serve as the main intermediate hosts and carnivores serve as definitive hosts: dogs, cats, raccoon dogs, foxes, and wolves (Yang et al., 2012). Two species, *S. suihominis* and *S. bovihominis*, are zoonotically most important (Vangeel, 2007). Humans can spread Sarcocystis of 2 species: *S. suihominis* and *S. bovihominis*. These species are often found in the muscles of domestic and wild animals and in human intestine.

In recent years, the EU member states have been implementing national monitoring of sarcocystosis for evaluation of the relevance of these parasites to human health (EFSA, 2007, 2010). Only pigs and wild boars have been identified as intermediate hosts of *S. suihominis* whereas cattle serve as intermediate hosts for *S. bovihominis*. These species of animals are links in the human food chain and should be included in observation systems in behalf of preserving human health (Taylor et al., 2007). It is assumed that zoonotic Sarcocystis are spread in most species of animals used for human food in Europe, yet their effects on human health have been only tentatively investigated due to the lack of data (Fayer, 2004).

According to the available research data, the intensity of Sarcocystis infection in game animals used for food is high and in some countries even exceeds 90% (Spickschen, Pohlmer, 2002; Goldova et al., 2008). It is rather widespread in Cervidae (roes, deer and elks) among which their prevalence exceeds 80% (Dahlgren, Gjerde, 2007). In Europe and North America, the prevalence of Sarcosporidia infection in roes and red deer is very high and often exceeds 80% (Dahlgren, Gjerde, 2010). In Norway, the prevalence of sarcocystosis in deer has been determined to be 100% (Dahlgren, Gjerde, 2007). In Poland, 24.7% of wild boars, 88.7% of roes and 94.3% of deer were found infected with sarcocystosis (Tropilo et al., 2001). In Slovakia, the prevalence of sarcocystosis in dogs accounts for 87% (Hvizdošova, Goldova, 2009). The German Karlsruhe Veterinary Service (BfR Stellunghahme No. 026/2008) has carried out molecular biological research and determined that almost 100% of wild fauna (roes and deer) had Sarcocystis DNA. Examination of 364 wild boars in 2010 in Latvia showed that the prevalence of sarcocystosis in these animals amounted to 14.3% (Medne et al., 2010).

In Lithuania, the incidence of sarcocystosis in wild fauna was periodically investigated in 1981–1986 (Arnastauskienė, 1989) and 1995–2002 (Grikienienė et al., 2001; Malakauskas et al., 2002; Kutkienė, 2002). According to the data obtained by Lithuanian scientists on more than 100 cloven-hoofed, the prevalence of Sarcocystis parasite infection varied from 80% to 90%. According to the most recent data described by Prakas (2012), the highest prevalence of infection was determined in roes (97.6%) and high prevalence in wild boars (82.2%) and red deer (80.6%). Yet, these data were based on examination of a rather small number of samples. From the practical point of view, investigations of Sarcocystis cysts present in game and posing a potential risk to humans are very important (Prakas, 2012).

The aim of work was to assess the prevalence and intensity of Sarcosporidia infection in different groups of muscles in wild cloven-hoofed hunted for food in Lithuania.

Materials and methods

In conformity with EU Regulation No. 854/2004, a post-slaughter examination of game (wild boars, roes, elks, red deer, fallow deer, and sika deer) was conducted in the Lithuanian processing company for game animals in the period of 2013−2015. For accurate macroscopic diagnostics of sarcocystosis, the muscles of the esophagus, the diaphragm and the dorsum were thoroughly palpated and examined and the muscles of the mandible and the heart were palpated, examined and dissected. The remaining samples were obtained through cooperation with veterinary surgeons servicing hunting clubs or directly from hunters without post-slaughter examination. The samples were taken from the esophagus and the diaphragm of all the examined animals and from the heart of all the animals except red deer. When possible, the samples of other tissues – neck, mandible, dorsum and leg – were also taken. In total, 1,279 samples were microscopically examined by the compression method. The majority of the game samples were collected from the central part of Lithuania.

Compression method for detection of Sarcocystis in tissues. For microscopic examination, the tissue samples weighing 100 g were taken. Sarcocystis was diagnosed using the compression method and methylene blue stain (Bogush, 1976). A light microscope with magnification x40 was used. The muscles (1.0 g) were examined from all the muscle groups. The abundance of infection was evaluated following the method suggested by Bogush (1976) and Malakauskas et al. (2001): 1–10 Sarcocystis – low infection, 11–40 Sarcocystis – medium infection, and more than 40 Sarcocystis – intensive infection.

Statistical data analysis. The prevalence and abundance indices of Sarcocystis parasite infection in the examined game were calculated using Quantitative Parasitology 3.0 programme (Rozsa et al., 2000). The infection prevalence confidence intervals were calculated by Stern's test (Reiczigel, 2003). The confidence intervals for 95% of infection abundance mean, median and medium abundance were determined using the 'bootstrap' method. Aggregation of parasites, i.e. uneven distribution of parasites in hosts, was evaluated using the discrepancy index, which shows the discrepancy between the established and even distributions (Poulin, 1993). The Fisher's test was used for comparison of differences in the prevalence of infection between the examined samples. The differences in parasite abundance in the analysed animal species were evaluated by the Mood's test, i.e. by comparison of medians of infection. The differences were considered statistically significant when $P < 0.05$.

Results

The prevalence of Sarcosporidia (*Sarcocystis* spp.) infection in game meat varied markedly not only among animal species but also in different groups of muscles (Table 1). In the samples taken from Lithuanian game animals, Sarcocystis was absent from the neck muscles of wild boars only. The maximal 100% burden of Sarcocystis infection was found in all the examined esophagus, diaphragm and leg muscles of red deer and diaphragm and neck muscles of roes (Table 1).

The highest burden of Sarcocystis infection in elk muscles was found in the diaphragm (88.9%) and the lowest in the esophagus (70.4%). The abundance of Sarcocystis in esophagus muscles of elk was statistically significantly higher ($P < 0.05$) than those in diaphragm and heart muscles (mean 19.26; median 16) (Table 1). The samples of elk muscles contained a relatively small number of cysts (from 1 to 46). The high abundance of Sarcocystis was established in the muscles of one elk only. The detected Sarcocystis were of different size and shape (ribbon, cigar).

Examination of diaphragm, esophagus, neck and leg muscles of red deer showed a very high prevalence of Sarcocystis infection. In 3 muscle groups of 4 examined, 100% prevalence of Sarcocystis infection was determined (Table 1). Significant differences in the prevalence of infection between muscle groups in red deer were not detected ($P > 0.05$). The established abundance of infection in esophagus muscles was statistically significantly lower ($P < 0.0001$) compared with other groups of muscles between which the differences were insignificant ($P > 0.05$). Very high abundance means and medians (up to 138 and 145 respectively) of Sarcocystis infection were registered in diaphragm, neck and leg muscles of red deer. Intensive infection involved more than 90% of muscles in these groups. In these 3 groups of muscles, the number of intensive infection was statistically significantly higher ($P < 0.05$) than in diaphragm muscles.

The differences of the prevalence and the abundance of Sarcocystis infection in various muscle groups of sika deer were not statistically significant ($P > 0.05$). The prevalence of Sarcocystis infection of sika deer was very similar in all the examined muscle groups (Table 1). The highest mean value of Sarcocystis infection intensity (42.24) was found in heart muscles, whereas the highest intensity median (24) was established in esophagus muscles. The majority of cases of intensive Sarcocystis infection were found in heart, neck and leg muscles (33.3%, 31.1% and 28.9%, respectively). The number of Sarcocystis in heart and neck muscles was statistically significantly higher than in diaphragm muscles ($P < 0.05$).

The groups of muscles of roe showed a very high prevalence of infection. It ranged within 94.4%–100.0% (Table 1). The highest prevalence of infection was established in diaphragm muscles and the lowest in dorsal and leg muscles, yet no statistically significant differences of infection prevalence in various groups of muscles were established ($P > 0.05$). The highest infection intensity mean (102.97) was calculated for diaphragm muscles. Statistically significant ($P < 0.05$) infection intensity differences were established between esophagus and leg, diaphragm and leg, and neck and leg. Intensive infection in the examined muscle groups involved from 30.6% in leg muscles to 61.1% in neck and diaphragm muscles. Very high Sarcocystis burdens, i.e. > 500 sarcocystis per 1 g of muscles, were found in diaphragm muscles of 2 roes (694 and 569 cysts).

High prevalence of Sarcocystis infection was determined in esophagus, diaphragm, mandible and leg muscles of wild boars (83.5%–94.5%). In the samples of

neck muscles of wild boars, Sarcocystis cysts were absent (Table 1). In heart muscles, the observed prevalence of infection was statistically significantly lower ($P < 0.05$) than in esophagus, diaphragm, mandible and leg muscles; in leg muscles, it was lower than in esophagus and diaphragm muscles ($P < 0.001$). The examined muscles of wild boars contained up to 128 cysts. The highest infection intensity mean and median values were calculated for heart muscles (30.23 and 17, respectively).

By comparison of infection intensity in the examined muscles of wild boars, significant differences were determined between heart and leg ($P < 0.05$), heart and esophagus ($P < 0.0001$), heart and mandible ($P < 0.05$), esophagus and leg ($P < 0.05$), and diaphragm and leg ($P < 0.05$). Intensive Sarcocystis infections were found in all the examined muscle groups of wild boars with Sarcocystis burdens. Comparison of the groups of muscles statistically significantly ($P < 0.05$) showed that the greatest number of intensive infection (20.5%) occurred in diaphragm muscles of wild boars. No statistically significant differences were determined between diaphragm and heart muscles, yet it should be pointed out that only 28 heart samples from wild boars were examined.

Table 1. **Prevalence and intensity of Sarcocystis infection in the examined muscle groups (country of origin Lithuania)**

Muscle type	N	Prevalence (%)	Abundance range	Abundance mean	Abundance median
Elk					
Esophagus (*m. esophagi*)	27	70.37	1−46	19	16
Diaphragm (*m. phrenicus*)	27	88.89	2−29	10	7
Heart (*myocar-dium)*	27	74.07	1−34	11	7
Red deer					
Esophagus (*m. esophagi*)	31	100	15−101	50	47
Diaphragm (*m. phrenicus*)	54	100	11−208	138	145
Neck (*m. semispinalis capitis)*	54	92.59	24−199	129	136
Leg (*m. extensor carpi radialis*)	54	100	29−196	118	120
Sika deer					
Esophagus (*m. esophagi*)	45	88.89	1−78	29	27
Diaphragm (*m. phrenicus*)	45	84.44	3−71	24	20.5
Heart (*myocar-dium)*	45	84.44	3−134	42	24.5
Neck (*m. semispinalis capitis)*	45	91.11	1−125	35	24
Leg (*m. extensor carpi radialis*)	45	88.89	1−121	29	18
Roe					
Esophagus (*m. esophagi*)	36	97.22	2−317	99	84
Diaphragm (*m. phrenicus*)	36	100	2−694	103	62.5
Heart (*myocar-dium)*	36	97.22	1−261	61	36
Neck (*m. semispinalis capitis)*	36	100	1−256	66	56
Back (*m. longinsimus dorsi)*	36	94.44	3−137	49	45.5
Leg (*m. extensor carpi radialis*)	36	94.44	1−96	33	27.5
Wild boar					
Esophagus (*m. esophagi*)	127	93.70	1−103	16	11
Diaphragm (*m. phrenicus*)	127	94.49	1−128	26	15
Heart (*myocar-dium)*	28	46.43	5−102	30	17
Neck (*m. semispinalis capitis)*	28	0	-	-	-
Jaw (*m. masseter)*	127	88.98	1−78	13	10
Leg (*m. extensor carpi radialis*)	127	83.46	1−83	11	8

Discussion

The data obtained by comparison of wild boars, roes and elks showed no statistically significant differences of Sarcosporidia infection. The lowest burdens of Sarcocystis were found in red deer and elks, whereas the highest in roes. It was statistically significantly established that mean infection intensity in esophagus and heart muscles was higher compared with diaphragm muscles and dependence on the animal age (Malakauskas, Grikienienė, 2002). Comparison of the prevalence and the intensity of Sarcocystis infection in various groups of muscles of different animals yielded different results. In sika deer, elks, red deer and roes, there were no statistically significant infection prevalence differences between the groups of muscles. The highest prevalence of infection was established in diaphragm, esophagus and mandible muscles of wild boars. The abundance of Sarcocystis infection in the examined species of animals often showed dependence on the group of muscles. Only in sika deer, statistically significant differences of abundance of

infection in various groups of muscles were not observed. Analysis of the abundance of infection indices allows stating that the highest abundance in the investigated species of animals is characteristic of esophagus muscles followed by diaphragm and heart muscles. The lowest infection intensity was identified in leg and dorsum muscles. It can be associated with activity of blood flow in the mentioned muscle groups and distance from the digestive tract.

It is worth pointing out that the prevalence of Sarcocystis infection in elks was high, whereas the abundance of infection almost in all cases was statistically significantly lower than in other species of Cervidae ($P < 0.05$). The differences in infection prevalence in various muscle groups were statistically insignificant, probably due to small number of examined samples ($P > 0.05$).

Almost in all the examined samples, the mean value of the abundance of Sarcocystis infection exceeded the median. This can be explained by parasite aggregation phenomenon when the number of Sarcocystis cysts in many individuals tends to be relatively small and in a few individuals it is high. A converse phenomenon has been observed in red deer. Their infection mean was very close to the median value (the mean values in diaphragm, neck and leg muscles were lower than the median). The D index, indicating the aggregation of parasites, in muscle groups of red deer was low (from 0.17 to 0.26) and approaching 0, which implied a theoretically even distribution of the samples. Without further investigations, it is to date difficult to explain the results obtained on the red deer. Yet, it should be pointed out that the number of the samples of the muscle groups from red deer was small (54 samples of diaphragm, neck and leg muscles and 31 samples of esophagus). This might have influenced the obtained results.

It is assumed that the incidence of Sarcocystis in different groups of muscles often varies. For example, examination of 400 dromedaries (Arabian camels) in Iran showed statistically significant differences of *Sarcocystis* spp. burden in tongue, esophagus, heart and diaphragm muscles (Shekarforoush et al., 2006). Furthermore, Malakauskas and Grikienienė (2002) found considerably higher burdens of Sarcocystis in heart and esophagus muscles compared with diaphragm muscles of red deer, roes and wild boars. Investigations of the same groups of horse muscles carried out in Brazil showed that the highest burdens of Sarcocystis tended to occur in the tongue and the esophagus (Bonesi et al., 1999). Due to different investigation methods, the data of Sarcosporidia infection intensity obtained by various researchers are hardly comparable, yet, nevertheless, certain regularities can be observed. The Sarcosporidia in game hunted for food are in direct correlation with the human environment and the environment of other species of animals. Some varieties of Sarcocystis are pathogenic and their spread in domestic and wild animals may produce an adverse effect on animal health. There is a common opinion that the adverse effects of Sarcosporidia infection usually manifest in intermediate hosts, whereas pathogenic capacity depends on the *Sarcocystis* variety and abundance of infection (Prakas, 2012).

Conclusion

In conclusion, it was shown that Sarcocystis infection in muscles of game animals – roes, red deer, sika deer, wild boars and elks – was very common. It should be emphasised that the prevalence and the abundance of infection in the muscles of roes and red deer was very high. Meanwhile, high abundance of Sarcocystis infection was found in the muscles of sika deer and low abundance in wild boars and elks. Finally, the highest abundance of Sarcocystis infection in the investigated animals was found in the ruminants of the *Cervidae* family.

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