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Prevalence and intensity of *Sarcocystis* spp. infection in animals slaughtered for food in Lithuania

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Abstract: The exact prevalence of *Sarcocystis* spp. infection in animals slaughtered for food is unknown in Lithuania. Therefore, the present study was initiated to evaluate *Sarcocystis* spp. infection in the carcasses of cattle (n = 206), sheep (n = 61), pigs (n = 73) and horses (n = 72) raised in Lithuania for food. The prevalence and intensity of *Sarcocystis* spp. infection were assessed under light microscopy by analysing 1 g of stained and squashed muscle samples. All the investigated muscle types (oesophagus, diaphragm, heart, neck, jaw, back, leg and tongue) were found to have been infected with microcysts rather than with macrocysts. A high prevalence of infection was established in cattle (44.9–98.1%) and sheep (100%), whereas the prevalence of this infection in pigs (30.1–50.0%) and horses (34.7–63.9%) was considered to be moderate. Significant differences in the infection prevalence were detected in the majority of muscle groups of cattle and in some muscle groups of pigs and horses. Similarly, significant differences in the median (Md) intensity of infection were observed in the majority of the muscle groups of cattle (Md = 4–29) and sheep (Md = 21–73) and only in some muscle groups of pigs (Md = 4.5–16) and horses (Md = 1–3). Cases of intense infection (> 40 cysts in a sample) were relatively often detected in sheep (44.9%) and cattle (19.1%), and rarely in pigs (3.7%). Hence, based on the varying rates of infection in the examined samples, the infection was identified as being intense in sheep and cattle, moderate in pigs and low in horses.

Keywords: cattle; sheep; pigs; horses; infection prevalence; infection intensity

More than 200 zoonotic diseases are transmitted from animals to people. They include echinococcosis, trichinosis, cysticercosis, ascariasis and sarcocystosis (Frenkel and Smith 2003). The genus *Sarcocystis* (Apicomplexa: *Sarcocystidae*) consists of more than 190 species that are prevalent in mammals, birds and reptiles. Some *Sarcocystis* species are important pathogens of humans and domestic and wild animals. These parasites are characterised by an obligatory two-host life cycle, the formation of sarcocysts mainly in the muscles of the intermediate hosts and endogenous sporulation

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of oocysts in the intestine of the definitive hosts (Dubey et al. 2015a). Two *Sarcocystis* species, *S. suihominis* and *S. hominis*, are considered to be the most important species in the EU from a zoonotic perspective (Vangeel et al. 2007). Pigs and boars are intermediate hosts of *S. suihominis*, whereas cattle are intermediate hosts of *S. hominis*. Furthermore, a further species, *S. heydorni* with a cattle-human life cycle has recently been described (Dubey et al. 2015b). Identification of the presence of pathogenic *Sarcocystis* spp. in meat consumed by humans should be included in the monitoring systems seeking to protect human health (Dubey and Odening 2001; Taylor et al. 2010).

Investigations into the distribution of sarcocystosis in cattle, pigs, sheep and horses slaughtered for food have been carried out at different times and in different countries in Europe, North and South America, Asia, Australia and Africa (Dubey et al. 2015a). *Sarcocystis suihominis* in pigs has been identified in Europe, India and Japan (Prasanth 1995; Saito et al. 1998; Dubey et al. 2015a). In India, a high prevalence of *S. suihominis* in pigs and humans is a result of unhygienic rearing and slaughtering of pigs, and the peculiarities related to the handling and consumption of pork (Chhabra and Samantaray 2013). The most recent investigations show a high percentage of *S. hominis* infection in beef in Germany, Belgium and Italy (Domenis et al. 2011; Vangeel et al. 2007; Vangeel et al. 2013; More et al. 2014).

In Lithuania, *Sarcocystis* spp. infection in domestic animals was studied more than 20 years ago (Grikieniene 1994). It was established that 90.6% of cattle and 34.7% of pigs reared on large farms were infected, whereas a significantly higher infection prevalence of 81.5% was determined in pigs raised on small farms. It should be noted that material for this investigation was only collected in western and southern Lithuania. No studies on *Sarcocystis* spp. infection in sheep and horses slaughtered for food have been conducted in Lithuania.

Although the prevalence of *Sarcocystis* infection in economically important domestic animals has been extensively investigated, there is still a lack of data on the extent of infection intensity and differences in infection prevalence and intensity by muscle type. The objective of the present study was to evaluate the prevalence and intensity of *Sarcocystis* infection in various muscle groups in cattle, sheep, pigs and horses slaughtered for food in Lithuania.

MATERIAL AND METHODS

Meat samples were collected and examined between 2012 and 2014. A pre-slaughter and post-slaughter examination of the animals (cattle, sheep, horses, pigs) was performed based on Regulation (EC) No. 854/2004 in the slaughterhouses of meat production companies. The post-slaughter examination of the carcasses of 2018 cattle, 475 sheep, 3069 pigs and 370 horses was carried out and no macroscopic lesions specific to sarcocystosis were detected. Subsequently, muscle samples of 206 cattle, 61 sheep, 73 pigs and 72 horses were examined microscopically (Table 1). All the examined animals were raised exclusively in Lithuania and were collected from the country's main two slaughterhouses, where animals are delivered from all regions of the country. Samples of oesophagus, diaphragm, heart, neck and jaw muscles were collected from all the animal species under study. Additionally, the back muscles from cattle and sheep, the leg muscles from pigs and the tongue muscles from horses were taken for examination (Table 1). The presence of sarcocysts was detected by means of a compressor-microscope method. In each case, 28 oat-size pieces (~ 1 g) of muscle were cut off, stained with 0.2% methylene blue solution, clarified with 1.5% acetic acid, pressed in a glass compressor and examined under a light microscope at 40 × magnification. In accordance with Malakauskas et al. (2001), the samples were divided

Table 1. Number of *Sarcocystis* spp. (infected/total investigated) in different muscles of domestic animals examined in Lithuania

Muscle type	Cattle	Sheep	Pigs	Horses
Oesophagus (*m. esophagi*)	202/206	61/61	33/71	41/72
Diaphragm (*m. phrenicus*)	171/206	61/61	36/72	33/72
Heart (*myocardium)*	187/206	61/61	22/73	25/72
Neck (*m. semispinalis capitis)*	187/206	61/61	22/73	45/72
Jaw (*m. masseter)*	159/206	61/61	29/64	26/72
Back (*m. longinsimus dorsi)*	87/194	61/61	–	–
Leg (*m. extensor carpi radialis*)	–	–	22/73	–
Tongue (*m. linguae)*	–	–	–	46/72
Overall	993/1224	366/366	164/426	216/432

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into four groups by the number of sarcocysts in 28 oat-size muscle sections, i.e. 0 cysts – no infection, 1–10 cysts – low infection, 11–40 cysts – moderate infection and > 40 cysts – intense infection.

The statistical analysis of *Sarcocystis* spp. infection prevalence and intensity was performed using the Quantitative Parasitology 3.0 software (Rozsa et al. 2000). In order to compare the quantitative indicators of *Sarcocystis* infection in cattle, sheep, pigs and horses, the infection data from the oesophagus, diaphragm, heart, neck and jaw were combined into one dataset. Confidence intervals for the infection prevalence were calculated using the Sterne or Wald methods (Reiczigel 2003). The unconditional exact test was performed to compare differences in the prevalence of infection (Reiczigel et al. 2008), while differences in infection intensity were evaluated comparing the median number of sarcocysts and using Mood's median test. Differences in variables were considered to be statistically significant when $P < 0.05$.

RESULTS

In the present study no macroscopic *Sarcocystis* spp. cysts were found, whereas microcysts were observed in all the muscle groups examined, i.e. the oesophagus, diaphragm, heart, neck, jaw, back, leg and tongue (Table 1). The highest *Sarcocystis* infection prevalence (100%) was found in sheep, whereas in cattle (44.9–98.1%), pigs (30.1–50.0%) and horses (34.7–63.9%) infection prevalence varied considerably depending on the muscle type (Table 2, Figure 1). A high infection prevalence (> 77%) was recorded in the majority of the mus-

Table 2. Prevalence and intensity indicators of sarcocyst infection in different muscles of domestic animals

Species	Prevalence and intensity indicators	Muscle type							
		oesophagus (a)	diaphragm (b)	heart (c)	neck (d)	jaw (e)	back (f)	leg (g)	tongue (h)
Cattle	prevalence (%)	98.1	83.0	90.8	90.8	77.2	44.9	–	–
	differences in prevalence ($P < 0.05$)	b c d e f	a c d f	a b e f	a b e f	a c d f	a b c d e	–	–
	intensity	1–3000	1–216	1–251	1–914	1–801	1–31	–	–
	median	29.0	11.0	16.0	13.0	6.0	4.0	–	–
	differences in intensity ($P < 0.05$)	b c d e f	a c e f	a b e f	a e f	a b c d	a b c d	–	–
Sheep	prevalence (%)	100	100	100	100	100	100	–	–
	differences in prevalence ($P < 0.05$)	No	No	No	No	No	No	–	–
	intensity	6–1992	6–314	1–122	7–679	4–112	1–86	–	–
	median	73.0	34.0	24.0	51.0	21.0	17.0	–	–
	differences in intensity ($P < 0.05$)	b c e f	a f	a d f	c e f	a d	a b c d	–	–
Pigs	prevalence (%)	46.5	50.0	30.1	30.1	45.3	–	30.1	–
	differences in prevalence ($P < 0.05$)	c d g	c d g	a b	a b	No	–	a b	–
	intensity	1–72	1–63	2–46	1–34	2–52	–	1–19	–
	median	16.0	11.5	9.5	8.0	14.0	–	4.5	–
	differences in intensity ($P < 0.05$)	g	g	No	No	g	–	a c e	–
Horses	prevalence (%)	56.9	45.8	34.7	62.5	36.1	–	–	63.9
	differences in prevalence ($P < 0.05$)	c e	h	a d h	c e	a d h	–	–	b c e
	intensity	1–24	1–6	1–5	1–5	1–9	–	–	1–14
	median	3.0	2.0	2.0	1.0	2.0	–	–	2.0
	differences in intensity ($P < 0.05$)	c d e	No	a	a h	a	–	–	d

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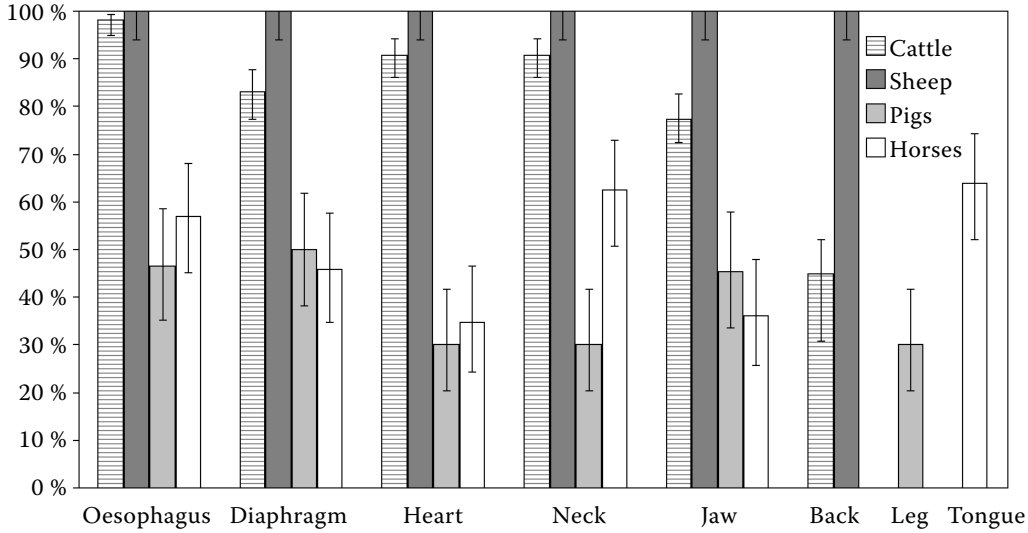

Figure 1. Prevalence of *Sarcocystis* infection (%) in different muscles of domestic animals. Error bars represent 95% CI

cles of cattle, except for back muscles. In horses and pigs, however, the infection prevalence values were lower and exceeded 50% only in the tongue, neck and oesophagus muscles of horses. In cattle, the infection prevalence was largely dependent on the muscle group, and no statistically significant differences in the prevalence of infection were observed between the diaphragm-jaw and the heart-neck pairs of muscles, while significant differences in the infection prevalence by muscle group were established in seven muscle pairs of horses and six muscle pairs of pigs out of 15 compared pairs. It should be noted that the highest infection prevalence values in each species were observed in different types of muscles, i.e. in cattle it was in the oesophagus, heart and neck muscles, in pigs it was in the diaphragm, oesophagus and jaw muscles and in horses it was in the tongue, neck and oesophagus muscles. Having pooled the infection prevalence data obtained from five muscle groups (oesophagus, diaphragm, heart, neck and jaw), a high infection prevalence was established in sheep (100%) and cattle (88.0%), whereas in horses (47.2%) and pigs (40.2%) it was considered to be moderate. Finally, statistically non-significant differences in infection prevalence were established between horses and pigs (Table 3).

Two different patterns in the relationship between *Sarcocystis* infection intensity and the type of muscles in the examined domestic animal species were revealed. In most cases infection intensity in cattle (the median (Md) values of infection intensity varied from four to 29) and in sheep (Md = 21–73) depended on the type of muscle examined. However, in pigs (Md = 4.5–16) and horses (Md = 1–3) such a relationship was only identified when comparing several pairs of muscle groups (Table 2, Figure 2). It should be pointed out that the highest median values of infection intensity in all four domestic animal species analysed were calculated in the oesophagus muscles, whereas in other muscle groups these values were similar.

When infection intensity was conditionally grouped into four categories (Figure 3), the significantly highest proportion of intense infection (> 40 cysts in a sample) in cattle was detected in the oesophagus (39.3%); intense infection was also found relatively often in the heart (24.3%) and neck (18.9%) muscles, rarely detected (< 10%) in the diaphragm and jaw muscles and not observed at all in

Table 3. Comparison of sarcocyst infection prevalence and intensity in domestic animals combining data obtained in five muscle groups (oesophagus, diaphragm, heart, neck and jaw)

Species	*N*	Prevalence (%)	Differences in prevalence ($P < 0.05$)	Median of intensity	> 40 cysts in sample (%)	Differences in intensity ($P < 0.05$)
Cattle (a)	1030	88.0	b c d	14.0	19.1	b d
Sheep (b)	305	100.0	a c d	34.0	44.9	a c d
Pigs (c)	353	40.2	a b	11.5	3.7	b d
Horses (d)	360	47.2	a b	2.0	0	a c d

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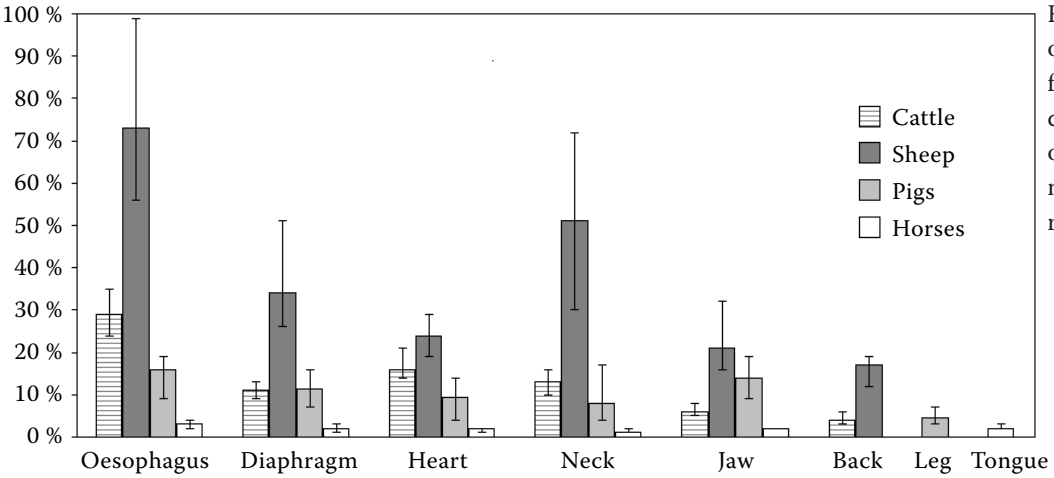

Figure 2. Median of *Sarcocystis* infection intensity in different muscles of domestic animals. Error bars represent 95% CI

back muscles. Meanwhile, intense infection was established in all the examined muscle groups of the sheep, i.e. 70.5% in the oesophagus, 47.1% in the neck, 45.9% in the diaphragm, 29.5% in the jaw, 23.0% in the heart and 14.8% in the back. In sheep, the highest number of cases of intense infection was found in the oesophagus, and a slightly lower level was found in neck muscles. In pigs, low or moderate infection, excluding uninfected samples, was most often identified, while intense infection did not exceed 7% and no cases of intense infection were detected in the neck and leg muscles. The number of sarcocysts found in the examined muscle samples of horses was low, ranging from one to 24. Also, taking into account the very small median values of infection intensity (1–3), infection intensity in horses was evaluated as low. It is worth mentioning that an extremely large number of sarcocysts, i.e. 100 or more cysts in 28 oat-size pieces of muscles (~ 1 g), was detected in some cattle and sheep samples. Specifically, an extremely high infection intensity was determined mostly in the oesophagus (21.8%) and neck (12.6%) muscles of cattle and in the oesophagus (36.1%), diaphragm (21.3%) and neck (19.7%) muscles of sheep. Furthermore, several instances (< 6%) of extremely high infection were recorded in the heart and jaw muscles of cattle and sheep, as well as in the diaphragm muscles of cattle. Moreover, more than 500 cysts in 28 muscle sections were detected in 15 oesophagus samples, three neck samples and one jaw sample of cattle, as well as in eight oesophagus samples and two neck samples of sheep. Of all the examined animals, a 24-month-old bull with 3000, 207, 214, 197, 176 and nine cysts in 28 sections of the oesophagus, diaphragm, heart, neck, jaw and back muscles, respectively, was the most heavily infected.

In conclusion, when the infection data obtained in five muscle groups were combined, median

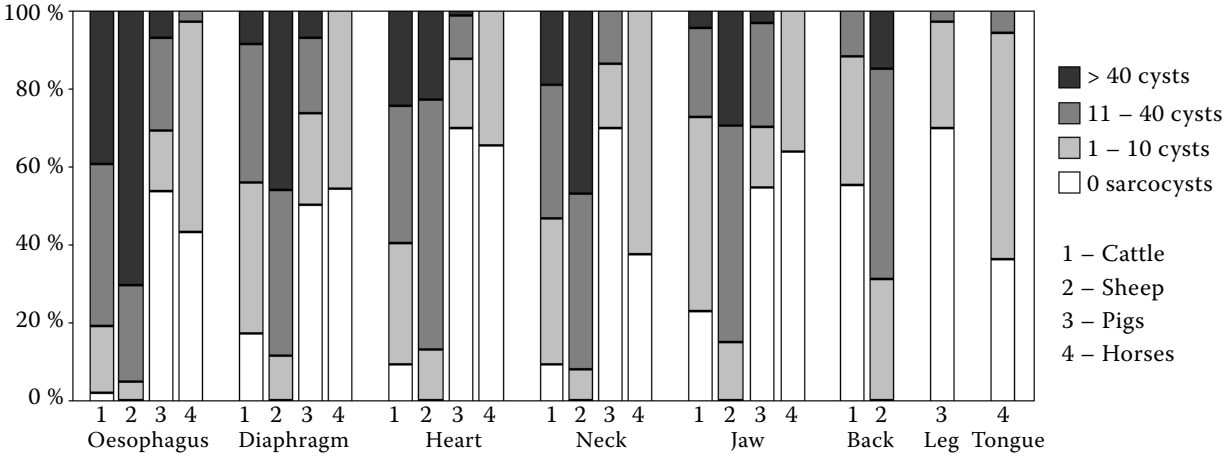

Figure 3. Distribution of *Sarcocystis* infection intensity in different muscles of domestic animals. Low infection: 1–10 cysts; moderate infection: 11–40 cysts; intense infection: 41 or more cysts

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numbers of sarcocysts detected in the investigated animals ranked in the following ascending order: horses (2) < pigs (11.5) < cattle (14) < sheep (34) (Table 3). Differences in median intensity were insignificant when comparing pigs and cattle only. Nevertheless, very similar median intensity values were detected in most examined muscle groups in cattle and pigs; significantly more cases of intense infection were found in cattle (19.1%) than in pigs (3.7%). Meanwhile, the proportion of intense infection cases in sheep was as high as 44.9%. Hence, the infection intensity of sarcocysts was assessed to be high in sheep and cattle, moderate in pigs and low in horses.

DISCUSSION

The results of *Sarcocystis* infection prevalence reported in the present work are in agreement with those of similar studies. High infection prevalence rates in cattle have been obtained in Lithuania and other countries around the world. For instance, an infection prevalence of 100% in cattle was recorded in Brazil (da Silva et al. 2002), Iran (Nourollahi-Fard et al. 2009), the USA and Argentina (More et al. 2011). In Russia, infection prevalence varied between 82.2% and 100% (Serdobijceva and Sidorkin 2011; Abakumov 2012), and in Egypt it amounted to 60–100% (Sayed et al. 2008; Nahed et al. 2014). Lower infection prevalence has been determined in Australia (52%) (Savini et al. 1992). Research data also show a relatively high prevalence of *Sarcocystis* infection in sheep. In Saratov (Russia), 100% of sheep older than six months were infected with sarcocysts (Serdobijceva and Sidorkin 2011). In Tabriz (Iran), macrocysts were found in 5.6% of the diaphragm and 2.7% of the oesophagus muscles, whereas microcysts were detected in all the sheep examined (Mirzaei and Rezaei 2016). The extent of *Sarcocystis* infection prevalence in pigs varies from low to high depending on the country and the year of research. For instance, only 16, 18 and 27% of slaughtered pigs were infected in Japan (Omata et al. 1993), USA (Dubey and Powell 1994) and the Philippines (Claveria et al. 2001), respectively, whereas high infection prevalence rates exceeding 50% have been determined in India (Chhabra and Samantaray 2013). Considerable variations in the infection prevalence in pigs have been observed in different areas of China (8–80%) and Russia (5–73%) (Usakova and Silnikova 2001; Serdobijceva and Sidorkin 2011; Yang et al. 2012). The prevalence of infection also varies greatly in horses, i.e. from 6% in Japan to 93% in Mongolia (Dubey et al. 2015a).

Currently there are only a few reports available on the prevalence of *Sarcocystis* infection in domestic animals reared in European countries, including the central and eastern parts of the continent. Infection prevalence in cattle ranges between 66.0% and 78.1% in Hungary (Hornok et al. 2015) and between 57.5% and 83.6% in Estonia (Lassen and Talvik 2009). In Italy, the infection prevalence in cattle varies between 78.1% and 91% (Domenis et al. 2011; Chiesa et al. 2013) and in Portugal from 64.6% to 100% (Goncalo 2014). In France, infection prevalence varies from 90% to 100% (Sertin et al. 2014). A lower infection prevalence in cattle (26.4%) has been determined in Germany (More et al. 2014). An infection level exceeding 50% has been determined in slaughtered pigs in Romania (Tabaran et al. 2013) and Germany (Damriyasa et al. 2004).

As concerns the prevalence of different *Sarcocystis* species, *S. cruzi* accounts for the majority of all identified species of this genus in cattle. In France, *S. cruzi* and *S. hominis* were found in 80% and 69% of samples, respectively (Sertin et al. 2014). In Germany *S. cruzi, S. sinensis, S. hirsuta* and *S. hominis* were found in 52, 37, 6.6 and 6.2% of investigated bovine samples, respectively (More et al. 2014). The study performed in Hungary shows that *S. cruzi, S. hominis* and *S. sinensis* are the most prevalent species, having been found in 64, 19 and 17% of investigated cattle (Hornok et al. 2015).

Summarising the available data, the prevalence of *Sarcocystis* infection in cattle and sheep is higher than that in pigs and horses. However, it should be emphasised that in examining the prevalence of *Sarcocystis* infection in domestic animals, different sarcocyst identification techniques, i.e. muscle squash, digestion, histology, serology or PCR, have been used. Therefore, it is difficult to perform a detailed analysis and comparison of the prevalence data. Moreover, the infection prevalence might vary considerably depending on muscle group. In this study, significant differences in the prevalence of infection were established between some pairs of muscle groups of cattle, pigs and horses.

Statistically significant differences in the intensity of *Sarcocystis* infection were detected in the majority of muscle group pairs of cattle and sheep and in some muscle group pairs of pigs and

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horses. Thus far, the extent of *Sarcocystis* infection intensity in Lithuania has been considered to pose a potential risk to human and animal health. Furthermore, the very large number of sarcocysts found in some muscle samples of sheep and cattle in the present investigation are a cause for serious concern. An extremely large number of sarcocysts (100 and more cysts in one g of muscle sample) was detected relatively often in the oesophagus and neck muscles of cattle and in the oesophagus, diaphragm and neck muscles of sheep.

At the present time, it is difficult to calculate economic losses caused by microscopic sarcocysts in domestic animals, since it is difficult to diagnose clinical sarcocystosis and evaluate loses sustained due to poor feed efficiency, failure to row, reduced milk and wool production and reproductive problems (Dubey et al. 2015a). Thus far, five *Sarcocystis* species have been identified in cattle (*S. cruzi, S. heydorni, S. hirsuta, S. hominis* and *S. rommeli*), four in sheep (*S. tenella, S. gigantea, S. arieticanis* and *S. medusiformis*), three in horses (*S. neurona, S. bertrami* and *S. fayeri*) and three in pigs (*S. miescheriana, S. suihominis* and *S. porcifelis*, although the taxonomic status of the last species is still uncertain) (Dubey et al. 2015a; Dubey et al. 2015b). Cattle and pig sarcocystosis are important from a zoonotic perspective since humans are the final hosts of *S. hominis, S. heydorni* and *S. suihominis* (Fayer 2004; Li et al. 2007; Bucca et al. 2011; Dubey et al. 2015b). Furthermore, *S. cruzi* and *S. tenella* can cause acute sarcocystosis in cattle and sheep, respectively. In contrast, *Sarcocystis* spp. found in pigs and horses in Europe are mostly mildly pathogenic to their intermediate hosts. According to the data from the present study, high *Sarcocystis* infection prevalence and intensity were established in sheep and cattle in Lithuania. Although sheep sarcocystosis is assumed to entail no direct risk to human health, it incurs economic losses due to the lower quality of meat. The infected animals suffer from weight loss, anaemia, reduced production of milk and wool, an affected central nervous system and abortions (Dubey et al. 2015a). Since the results of the present study indicate high *Sarcocystis* infection intensity in all the sheep muscles examined, a potential threat to human health cannot be excluded. Moderate sarcocyst infection prevalence and intensity in pigs and moderate prevalence and low intensity in horses were estimated in this work. At the present time it is not possible to clarify precisely whether pig sarcocystosis could cause health problems for humans in Lithuania, since the prevalence of *S. suihominis* has not been investigated. In the meantime, it is assumed that horse sarcocystosis is a matter of low concern in Lithuania.

Some *Sarcocystis* species are pathogenic and the distribution of such parasites among domestic animals can negatively affect human and animal health. Thus, the primary focus should be on the monitoring, control and prevention of *Sarcocystis* infection, as well as on the development of new diagnostic methods to identify the presence of pathogenic *Sarcocystis* species in raw meat and processed meat products (Chiesa et al. 2013). Significant differences in *Sarcocystis* infection prevalence and intensity in various muscle groups of cattle, sheep, pigs and horses were identified in the present study. This information could prove useful for undertaking a further evaluation of meat products that might pose a threat to human health due to *Sarcocystis* infection.

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