

# Protozoan parasites from genus *Sarcocystis* and their investigations in Lithuania

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The representatives of the genus *Sarcocystis* are cyst forming coccidian protozoa parasites broadly prevalent in mammals, birds and reptiles. *Sarcocystis* parasites are characterized by an obligatory prey-predator two-host life cycle. Currently, over 220 *Sarcocystis* species are known. Some of *Sarcocystis* species are pathogenic organisms dangerous to humans, domestic and wild animals. A harmful effect of *Sarcocystis* mainly occurs in intermediate hosts and depends on the species of parasite, infection intensity and localization in the body. In Lithuania, *Sarcocystis* species forming sarcocysts in rodents, even-toed ungulates and birds were mainly studied. The ecology and diversity of *Sarcocystis* parasites were investigated using traditional morphological, DNA analysis methods and transmission experiments. Lithuanian scientists have described and named five *Sarcocystis* species, i. e. *S. rodentifelis*, *S. wobeseri*, *S. cornixi*, *S. anasi*, *S. albifrons*; for the first time proved *Sarcocystis rileyi* infection in Europe; established definitive hosts of some *Sarcocystis* species.

**Key words:** *Sarcocystis*, pathogenicity, diversity, investigations in Lithuania

## INTRODUCTION

The members of the genus *Sarcocystis* are intracellular cyst forming coccidian protozoa parasites, which are characterized by an obligatory heteroxenous prey-predator two-host life cycle. Herbivores and omnivores usually serve as intermediate hosts and carnivores as definitive hosts of these parasites. Asexual multiplication occurs in intermediate hosts, and after merogony sarcocysts in muscle tissues are formed. The sexual phase with the formation of oocysts/sporocysts takes place in the small intestine of definitive hosts. Oocysts sporulate in the lamina propria (Mehlhorn, Heydorn, 1978).

The genus *Sarcocystis* is represented by the biggest number of species in the family Sarcocystidae (Tenter, Johnson, 1997). In the last taxonomical review of this genus, the list of 189 spe-

cies was proposed (Odening, 1998). At present, their number is estimated to be over 220 species. In general, *Sarcocystis* species are found in mammals, birds and reptiles. The only exception is *S. salvelini*, whose intermediate host is brook trout (*Salvelinus fontinalis*) belonging to the family Salmonidae (Fig. 1). The majority of *Sarcocystis* species are described in the intermediate host, and a complete life cycle is ascertained to less than half of them. Several combinations of intermediate/definitive hosts are known and they are put in order from the most common: mammals/mammals, mammals/reptiles, reptiles/reptiles, mammals/birds, birds/mammals, birds/birds.

The objectives of this paper are to characterize the main biological features of parasites from the genus *Sarcocystis*, to overview their diversity and parasitism in different groups of organisms, to summarize investigations carried out in Lithuania.

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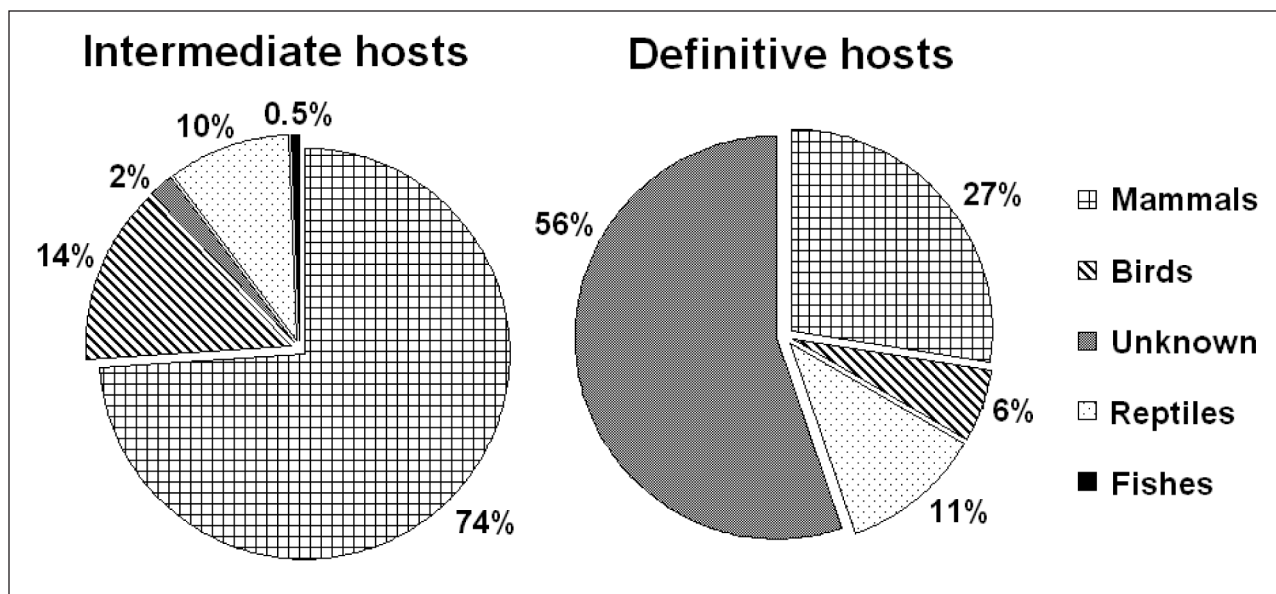


Fig. 1. Intermediate and definitive hosts of *Sarcocystis* species. Pie charts were drawn using summarized data from literature

### The biology of *Sarcocystis*

**Life cycle characteristic of *Sarcocystis*.** The definitive host becomes infected with *Sarcocystis* parasites by ingesting infected tissues containing mature sarcocysts (Fig. 2). After gametogony and sporogony, two sporocysts containing four sporozoites are formed in the small intestine. Sporocysts are passed into the environment through faeces. For most *Sarcocystis* species oocysts/sporocysts are first shed in the faeces between 7 and 14 days after ingesting sarcocysts. The spreading of oocysts/sporocysts usually lasts up to several months. Ingestion of sporocysts and oocysts through contaminated food or water is virtually the only way of transmission of these parasites to the intermediate host. The number of merogony generations and the type of infected host cells may vary depending on species of *Sarcocystis*. After merogony, sarcocysts with mature cystozoites are usually formed in striated muscles of the heart, tongue, esophagus, diaphragm, skeletal muscles, rarely in CNS, in gut, in Purkinje fibres of the heart. The structure of sarcocysts, especially characteristic of cyst wall, is the main morphological diagnostic criteria of *Sarcocystis* species. Sarcocysts vary in size and shape depending on the species of the parasite, maturation of the cyst and the type of the host cell. Sarcocysts generally become infectious at about 75

DPI, but there is a considerable variation among the species of *Sarcocystis* (Dubey et al., 1989).

Usually representatives of the genus *Sarcocystis* are characterized by a diheteroxenous life cycle, i. e. different animal species serve as an intermediate and final host. It was established that several *Sarcocystis* species (*S. atlanticae*, *S. dugesii*, *S. gallotiae*, *S. stehlinii*, *S. simonyi*, *S. muris*, *S. rodentifelis*, *S. cymruensis*) have a dihomoxenous life cycle, when sexual and asexual multiplication of parasites may be completed in the same host species (Matuschka, Bannert, 1987; Matuschka, 1988; Matuschka, Bannert, 1989; Bannert, 1992; Griekienienė, Kutkienė, 1998; Koudela, Modrý, 2000; Hu et al., 2011).

**Host specificity of *Sarcocystis*.** The majority of *Sarcocystis* species are strictly specific to the intermediate host, i. e. one *Sarcocystis* species can parasitize only one intermediate host species. The specificity of these parasites to the final host is considerably smaller. It is known that most of *Sarcocystis* species transmitted through canids cannot be transmitted through felids and vice versa. The only exception of this consistent pattern is *S. wenzeli* from chicken, whose definitive hosts are dogs or cats (Odening, 1997). Using morphological, immunological, genetic studies and clinical research methods it was ascertained that *S. neurona* has the

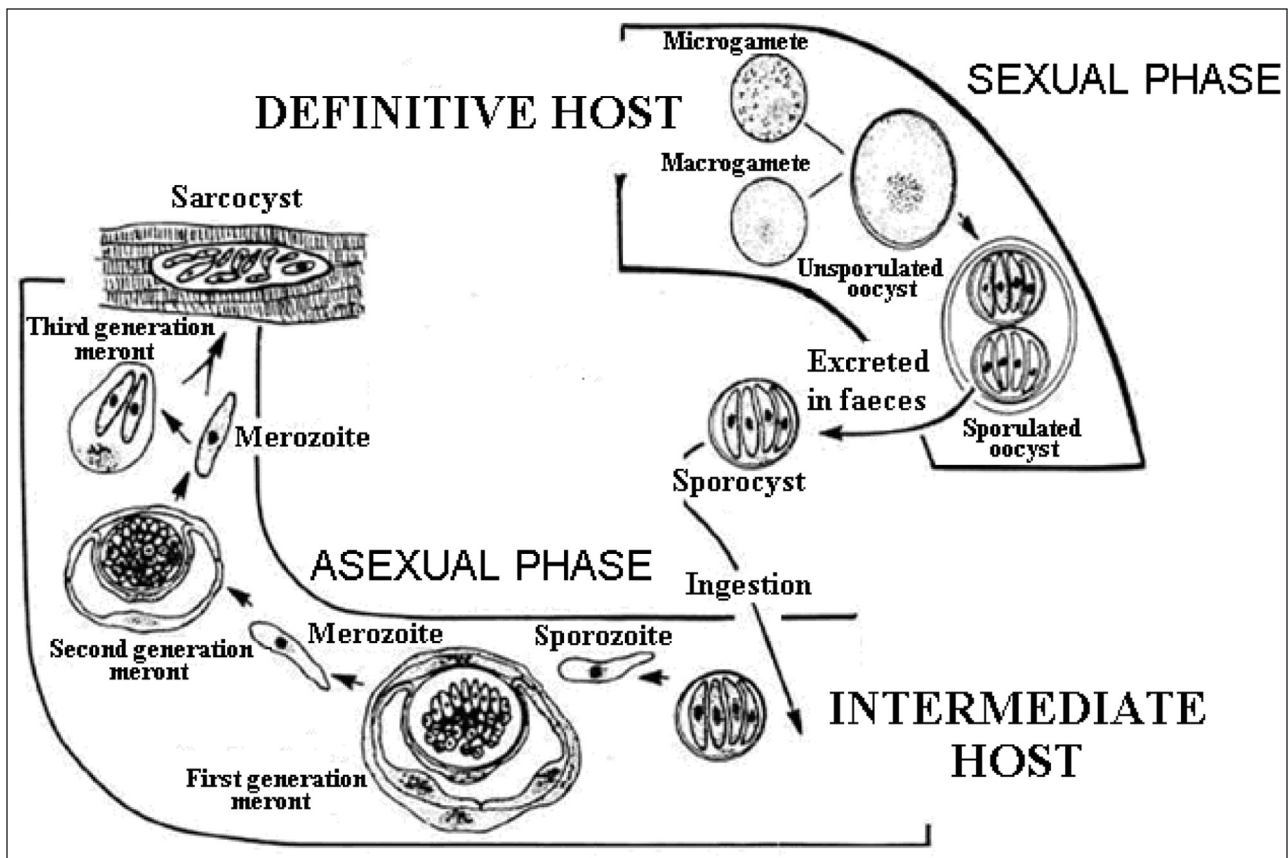


Fig. 2. Generalized life cycle of *Sarcocystis* species (Dubey et al., 1989)

lowest intermediate host specificity and can parasitize at least ten taxonomically distant animal species (Carlson-Bremer et al., 2012). Using cross-transmission experiments and DNA investigations it was showed that *S. falcatula* forms sarcocysts in the birds of five different orders (Box et al., 1984; Wünschmann et al., 2010). Morphologically similar sarcocysts are frequently found in muscles of taxonomically related intermediate host species. Therefore, for evaluating intermediate host specificity, more comprehensive investigations are required (Dubey et al., 1989; Wesemeier, Sedlaczek, 1995; Odening, 1998). The combined morphological and DNA analysis results revealed that some species can parasitize at least two different intermediate hosts species (Yang et al., 2001a, 2001b; Jehle et al., 2009; Dahlgren, Gjerde, 2010; Kutkienė et al., 2010; Gjerde, 2012).

**Pathogenicity of *Sarcocystis*.** Some of *Sarcocystis* species are important pathogenic organisms dangerous to humans and livestock and causing disease called sarcocystosis. Harmful pathogenic

effects of these parasites mainly occur in intermediate hosts, and infection in definitive hosts is usually mild. The pathogenicity of *Sarcocystis* depends on the species of the parasite, infection intensity and localization in the body. Pregnancy, lactation, lack of food or other stresses may influence the severity of clinical sarcocystosis (Dubey et al., 1989; Fayer, 2004).

Sarcocystosis is usually mild in animals living under natural conditions and clinical signs are unnoticeable, but sometimes it can end in death of infected animals. There are no specific clinical symptoms characterizing this disease. The signs of sarcocystosis are various – weakness, weight loss, anemia, fever, edema, diarrhea, hemorrhage, muscle twitching, muscle atrophy, hair loss, increased salivation, decreased lactation and etc. Severe sarcocystosis of some *Sarcocystis* species can cause hepatitis, encephalitis, and encephalomyelitis. Due to *Sarcocystis* infection fertilized females may lose their foetus. It is assumed that *Sarcocystis* parasites indirectly affect the health of the foetus,

reinforcing harmful effects of other diseases (Dubey et al., 1989; O'Donoghue, Rommel, 1992; Dubey et al., 2003b).

### **Sarcocystis diversity and occurrence in intermediate and definitive hosts**

**Human sarcocystosis.** The cases of muscular sarcocystosis in humans are rarely recorded, mainly in tropical or subtropical regions, especially in Southeast Asia. A seroepidemiological investigation in West Malaysia showed that 19.7% of 243 persons had antibodies to *Sarcocystis* (Thomas, Dissanaik, 1978). Thus, sarcocystosis is common but rarely diagnosed infection in Malaysia. An outbreak involving 7 of 15 military personnel in Malaysia is the largest cluster case on record (Arness et al., 1999). The symptoms of muscular sarcocystosis are acute fever, musculoskeletal pain, bronchospasm, rash, cardiomyopathy, swelling, and eosinophilia. People become infected with unidentified *Sarcocystis* species. It is believed that humans are accidental intermediate hosts of *Sarcocystis*, acquiring infection by ingesting sporocysts excreted by predators of nonhuman primates (Fayer, 2004).

Humans serve as definitive hosts of two *Sarcocystis* species, *S. hominis*, whose intermediate hosts are cattle or water buffalos (*Bubalus bubalis*), and *S. suihominis*, whose intermediate hosts are pigs or wild boars (*Sus scrofa*). Consumption of raw beef or pork contaminated with sarcocysts of these species may cause nausea, loss of appetite, vomiting, stomach ache, bloat, diarrhea, dyspnea, and tachycardia. Of fecal specimens examined from children in Poland and Germany, 10.4 and 7.3% were found positive for intestinal sarcosporidiosis, respectively (Fayer, 2004). Meanwhile, in Lithuania *Sarcocystis* oocysts/sporocysts were present in excrements of 25 humans out of 1184 (2.1%) examined (Grikienienė, 1989b).

**Sarcocystis in mammals.** *Sarcocystis* species found in economically important domestic animals are the most comprehensively studied as a result of economic, social aspects and possible transmission to humans. Definitive hosts were determined almost for all *Sarcocystis* species parasitizing livestock. Moreover, their infection prevalence, pathogenesis, genetic and immunological characteristics were assessed. *Sarcocystis* infection prevalence in domestic mammals ranged from 10

to 100% and the highest prevalence was determined in cattle, sheep and goats. The final hosts of *Sarcocystis* species parasitizing domestic animals are canids, felids and humans. A number of *Sarcocystis* species found in domesticated mammals varies from one to six (Dubey et al., 1989; Odening, 1998).

In general, *Sarcocystis* species transmitted via canids are more pathogenic than those transmitted via other definitive hosts. Cattle, sheep, goats, pigs, alpaca (*Lama lama*) farmers lost millions because of reduced meat, milk, wool, production. Cattle or sheep meat containing grossly visible sarcocysts is not suitable for sale. Nevertheless, economic loss due to sarcocystosis, with rare exceptions has almost not been evaluated (O'Donoghue, Rommel, 1992).

*S. neurona*, *S. canis*, *S. cruzi*, *S. tenella*, *S. capracanis* are the most pathogenic species parasitizing wild and domestic mammals. *S. neurona* causes a very dangerous horse disease, equine protozoal myeloencephalitis. The symptoms of this disease are: ataxia, weakness, cramp or stiff gait, muscle atrophy, face nerve paralysis, head tilt, difficulty in chewing or ingesting food, dropped eyelid or lip, abnormal eye movements, back pain, posture changes, movement in a circle, frequent lying, seizures, prostration and death (Fritz, Dubey, 2002; Saville et al., 2002). Other intermediate hosts of CNS diseases agent *S. neurona* are: cats, ferrets (*Mustela putorius furo*), raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*), nine-banded armadillos (*Dasypus novemcinctus*), brown-headed cowbirds (*Molothrus ater*), sea otters (*Enhydra lutris*), harbor seals (*Phoca vitulina*), California sea lions (*Zalophus californianus*) (Dubey et al., 1991; Carlson-Bremer et al., 2012). Severe *S. canis* and *S. canis*-like infection leads to hepatitis in grizzly bears (*Ursus arctos horribilis*), American black bears (*Ursus americanus*), long-tailed chinchillas (*Chinchilla lanigera*), dogs, horses, California sea lions, Hawaiian monk seals (*Monachus schauinslandi*), striped dolphins (*Stenella coeruleoalba*) (Dubey et al., 2003b). *S. cruzi* from cattle, *S. tenella* from sheep and *S. capracanis* from goats can cause a variety of clinical signs, including anorexia, anemia, weight loss, abortion, hair loss, weakness, muscle inflammation, prostration, decreased milk production, salivations, neurological disorders and death (Dubey et al., 1989).

Investigations of wild animal *Sarcocystis* are relevant when evaluating infection prevalence, pathogenesis, host specificity, inter- and intra-genetic variability, deepening knowledge of infection mechanisms, life cycle models, and host-parasite evolutionary relationships. Furthermore, studies of *Sarcocystis* parasites in game animals are important due to potential threat to humans. For instance, diarrhea, nausea and vomiting symptoms because of eating roe deer meat intensely infected with sarcocysts were reported (Schultze, 1988). After analysis of *Sarcocystis* diversity in wild mammals, the largest numbers of *Sarcocystis* species were ascertained for even-toed ungulates and rodents (Odening, 1998). Predatory mammals are the most important distribution agents of *Sarcocystis* species and usually serve as definitive hosts. Moreover, they are also intermediate hosts of several *Sarcocystis* species (Dubey, Speer, 1991; Dubey et al., 1992; Odening et al., 1994; Dubey et al., 2010a).

**Investigation of *Sarcocystis* species found in domestic mammals in Lithuania.** In Lithuania, the first investigations of *Sarcocystis* infection in domestic animals, i. e. cattle and pigs, started in 1976 (Arnastauskienė et al., 1979). According to the data of the most comprehensive study carried out in the Klaipėda meat-packing plant, the prevalence of *Sarcocystis* infection in cattle and pigs was 90.6 (940 investigated) and 34.7% (1113 investigated), respectively (Griekienienė, 1994). A significantly higher infection prevalence and intensity was established in heart muscles of cattle compared with diaphragm and skeletal muscles, also in pig heart and diaphragm muscles compared with skeletal muscles. Much higher prevalence of this parasitosis was determined in pigs raised in individual small farms (81.5%; 207 infected out of 254 investigated). A different rate of infection prevalence could be explained by pig rearing conditions. In specialized pig farm complexes, movement freedom is restricted. There pigs are kept in stalls all year round, fed on combined fodder and slaughtered approximately at the age of eight months. Therefore, possibilities of infection with *Sarcocystis* sporocysts through fodders, water reservoirs are minimized.

J. Griekienienė (maiden name – Kazakauskaitė) exhaustively examined the morphological and cytochemical structure of *S. gigantea* parasitiz-

ing sheep (Kazakauskaitė, 1980a, 1980b, 1980c; Griekienienė, 1983). The third morphological type, i. e. interstitial cells, was distinguished within the cyst of *S. gigantea* and this type has been first discovered for the genus *Sarcocystis* (Kazakauskaitė, Sidorenko, 1980). Later it was confirmed that *S. gigantea* circulates between sheep and cats (Griekienienė, 1989a).

Meanwhile, sarcocysts were not detected in 555 rabbits examined (Kutkienė, Griekienienė, 1994). To our knowledge, *Sarcocystis* investigations in other domestic mammals in Lithuania have not been carried out.

**Investigation of *Sarcocystis* species found in rodents in Lithuania.** According to the data of the most comprehensive *Sarcocystis* study in rodents in Lithuania, 12.3% animals (96 out of 778) were infected with sarcocysts. Cysts were found in the leg muscles of the common rat (*Rattus norvegicus*), black rat (*Rattus rattus*), bank vole (*Clethrionomys glareolus*), common vole (*Microtus arvalis*), tundra vole (*Microtus oeconomus*) and field vole (*Microtus agrestis*) (Griekienienė et al., 2001). Furthermore, *Sarcocystis* spp. were detected in the yellow-necked mouse (*Apodemus flavicollis*) and striped field mouse (*Apodemus agrarius*) (Arnastauskienė, Griekienienė, 1993). The highest infection prevalence was determined in the common rat and black rat, 52.4 and 30.2%, respectively (Griekienienė et al., 2001). The prevalence of these parasites in voles ranged from 8.3 to 20.4% (Griekienienė, Mažeikytė, 2000; Griekienienė et al., 2001). Under a light microscope, *S. putorii* transmitted via members of the family Mustelidae was identified in the common vole and field vole. In the muscles of the field vole thin-walled *Sarcocystis* sp. microcysts without protrusions predominated. Three different morphological types of microcysts were distinguished in the bank vole, i. e. sarcocysts with a smooth cyst wall and large cystozoites; sarcocysts with hair-like protrusions and small cystozoites; sarcocysts having claw-shaped protrusions and large cystozoites. It is supposed that these morphologically different types of sarcocysts represent three separate *Sarcocystis* species that have not been yet named (Griekienienė, Mažeikytė, 2000). Meanwhile, sarcocysts detected in rats were identified as *S. rodentifelis* (Griekienienė et al., 2001).

According to the experimental data it was supposed that rodents might be intermediate

hosts and definitive hosts for the same *Sarcocystis* species (Griekienienė, Arnastauskienė, 1992). *S. rodentifelis*, whose definitive host is usually the cat, for the first time was described by a group of Lithuanian scientists (Griekienienė et al., 1993). Later, using transmission experiments it was proved that rats may serve as final hosts of this species because of cannibalism and coprophagy (Griekienienė, Kutkienė, 1998; Kutkienė, Griekienienė, 2003). Thus, Lithuanian investigators made a significant contribution to deepening our understanding of *Sarcocystis* parasites life cycles.

**Investigation of *Sarcocystis* species found in even-toed ungulates in Lithuania.** Investigations of wild even-toed ungulates hunted in Lithuania revealed that *Sarcocystis* cysts were found in the muscles of the wild boar, roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), sika deer (*Cervus nippon*) and moose (*Alces alces*).

In wild boars, *S. miescheriana* and *S. suihominis*, whose definitive hosts are canids and humans, respectively, were detected in Lithuania. Griekienienė and Senutaitė (1995) for the first time ascertained that the raccoon dog (*Nyctereutes procyonoides*) is one of *S. miescheriana* definitive hosts. It should be noted that thin-walled sarcocysts of *S. suihominis* are found in Lithuania considerably less frequently (infection prevalence amounted to 8.3%) than thick-walled sarcocysts of *S. miescheriana* (infection prevalence was 91.7%). *Sarcocystis* infection prevalence in the wild boar was very high in many cases and exceeded 80% (Arnastauskienė, 1989; Griekienienė, Senutaitė, 1995; Griekienienė et al., 2001; Kutkienė, Baleišis, 2001; Malakauskas et al., 2001; Malakauskas, Griekienienė, 2002).

According to morphometric characteristics of sarcocysts, three *Sarcocystis* species, *S. capreolicanis*, *S. gracilis*, *S. hofmanni*-like, were identified in the roe deer in Lithuania by light microscopy (Griekienienė et al., 2001; Kutkienė, Baleišis, 2001). *S. hofmanni*-like detected in the muscles of the roe deer was morphologically indistinguishable from *S. hofmanni*, described in the Eurasian badger (*Meles meles*) (Odening et al., 1994). Kutkienė (2001) characterized one more type of sarcocysts in the roe deer, which was very similar to *S. grueneri* from the reindeer (*Rangifer tarandus tarandus*). Analysis of 18S rRNA gene sequences indicated that there are at least two species previously

described as *S. hofmanni*-like (Prakas et al., 2008). Combining morphological and DNA analysis results, besides *Sarcocystis* species from roe deer previously detected in Lithuania, *S. oviformis* and *S. silva* were additionally identified (Prakas, 2011). The prevalence of *Sarcocystis* infection in the roe deer varied from 77.7 to 94.2% (Griekienienė et al., 2001; Kutkienė, Baleišis, 2001; Malakauskas et al., 2001; Malakauskas, Griekienienė, 2002).

Using light microscopy analysis *S. capreolicanis*-like, *S. hofmanni*-like and *Sarcocystis* sp. were diagnosed in the red deer hunted in Lithuania (Kutkienė, 2003). According to the morphological features, *S. capreolicanis*-like, *S. hofmanni*-like from the red deer were identical to *S. capreolicanis* and *S. hofmanni*-like parasitizing roe deer. Meanwhile *Sarcocystis* sp. from red deer was morphologically similar to *S. grueneri*. Based on 18S rRNA gene sequences, it was showed that *S. capreolicanis*-like from the red deer and *S. hjorti* is the same species (Prakas, 2011). The highest observed *Sarcocystis* infection prevalence in red deer in Lithuania was 84.2% (32 infected out of 38 investigated) (Griekienienė et al., 2001). Having examined 41 sika deers living in captivity in Kaunas district, 38 (92.7%) were positive for *Sarcocystis* spp. cysts (Malakauskas, Griekienienė, 2002).

*Sarcocystis* cyst in moose in Lithuania were firstly detected by Arnastauskienė and Kazlauskas (1984), however, species composition was not investigated. Later, macrocysts similar to *S. gigantea* and two morphological types of microcysts were characterized in moose and it is supposed that one type corresponds to *S. alceslatrans* (Griekienienė et al., 2001; Kutkienė, 2002). Furthermore, using cyst ultrastructure and DNA analysis results *S. hjorti* in moose was identified (Prakas, 2011). According to the most comprehensive moose *Sarcocystis* study in Lithuania, 83% (44 infected out of 53 investigated) infection prevalence was observed (Malakauskas, Griekienienė, 2002).

When summarizing the widest game even-toed ungulate *Sarcocystis* research carried out in Lithuania, the prevalence of *Sarcocystis* infection ranged approximately from 80 to 90% (Griekienienė et al., 2001; Kutkienė, Baleišis, 2001; Malakauskas et al., 2001; Malakauskas, Griekienienė, 2002). Statistically significant higher prevalence of *Sarcocystis* infection was established in the roe deer. While investigating game mammals in Lithuania, the highest

*Sarcocystis* intensity was recorded in diaphragm muscles of roe deer (a female over three years of age hunted in Panevėžys region), i. e. 400 sarcocysts were detected in 28 oath-size sections (~1 g) (Malakauskas, Griekienienė, 2002). Furthermore, in one section of some individuals of roe deer even up to 40 cysts were detected (Griekienienė et al., 2001). It should be noted that sarcocysts intensity in esophagus and heart muscles of investigated game species was higher than in the diaphragm. Also, higher prevalence and intensity parameters of *Sarcocystis* infection were observed in adult even-toed ungulates compared with juveniles, but significant differences between sexes were not detected (Malakauskas, Griekienienė, 2002). The cases of mix infection were observed in the roe deer, red deer and moose, when from one to four different morphological types of sarcocysts were found in one host (Kutkienė, 2001, 2002, 2003).

**Investigation of *Sarcocystis* species found in other wild mammals in Lithuania.** In the order Insectivora in Lithuania, *Sarcocystis* spp. microcysts were found only in the limb muscles of the common shrew (*Sorex araneus*), while the southern white-breasted hedgehog (*Erinaceus concolor*), European mole (*Talpa europaea*), Eurasian pygmy shrew (*Sorex minutus*) and Eurasian water shrew (*Neomys fodiens*) were negative for sarcocysts (Griekienienė, Mažeikytė, 2000; Griekienienė et al., 2001; Žasitytė, Griekienienė, 2002). Infection prevalence in common shrew reached up to 7.1% (Arnastauskienė, Griekienienė, 1993).

*Sarcocystis* infection prevalence in the European hare (*Lepus europaeus*) hunted in Lithuania was low (7.3%) (Arnastauskienė, Kazlauskas, 1984). Later, Kutkienė and Baleišis (2001) examined 15 hares, but did not find sarcocysts.

In the period of 1987–2000, in diaphragm and skeletal muscles of 70 examined mammalian predators: grey wolves (*Canis lupus*), red foxes (*Vulpes vulpes*), raccoon dogs, European pine martens (*Martes martes*), beech martens (*Martes foina*), least weasels (*Mustela nivalis*) and European polecats (*Mustela putorius*), *Sarcocystis* cysts were not found (Griekienienė et al., 2001; Kutkienė, Baleišis, 2001).

***Sarcocystis* in birds.** To date, 30 named *Sarcocystis* species forming sarcocysts in the muscle tissues of birds belonging to at least 13 avian orders are known (Kutkienė et al., 2012). Two *Sarco-*

*cystis* species were described in chickens, *S. wenzeli*, whose definitive hosts are dogs or cats, and *S. horvathi*, whose definitive host is still undiscovered (Odening, 1997). Species name was not given for sarcocysts found in other poultry species. In North America *Sarcocystis* macrocysts were detected in wild ducks, geese and other water birds (Erickson, 1940). These macrocysts were attributed to *S. rileyi* forming sarcocysts resembling rice grain. The striped skunk (*Mephitis mephitis*) was found to be a definitive host for this species (Cawthorn et al., 1981; Wicht, 1981). The shoveler (*Anas clypeata*) and the mallard (*Anas platyrhynchos*) serve as intermediate hosts for *S. rileyi*; however, it is considered that the circle of possible intermediate hosts is much wider (Dubey et al., 2003a, 2010b). *S. rileyi* has only a mild pathogenicity, but the meat of hunted birds infected with *Sarcocystis* macrocysts is not suitable for food (Chabreck, 1965; Dubey et al., 2003a). In ducks, low prevalence of *Sarcocystis* macrocysts was mostly recorded (Hoppe, 1976; Drouin, Mahrt, 1979; Costanzo, 1990; Fedynich, Pense, 1992).

Some highly pathogenic *Sarcocystis* species, *S. falcatula*, *S. calchasi*, *S. neurona*, parasitize birds. *S. falcatula* infect birds of order Passeriformes, Psittaciformes, Columbiformes, Strigiformes, Accipitriformes and the final host of this species is the Virginia opossum (*Didelphis virginiana*). *S. falcatula* meronts extensively develop in the blood vessels of birds up to 5.5 months and this determines severe pathogenicity of these parasites (Box et al., 1984; Smith et al., 1990; Wünschmann et al., 2010). *S. neurona* causing neurological diseases to mammals was recently identified in the brown headed cowbird (Mansfield et al., 2008). *S. calchasi* parasitizing the domestic pigeon (*Columbia livia* f. *domestica*) is transmitted by predator birds of the genus *Accipiter* (Olias et al., 2011). The clinical signs of *S. calchasi* infection are depression, polyuria, torticollis, opisthotonus, paralysis, trembling and death (Olias et al., 2009, 2010a, 2010b). Predatory birds are final hosts of several *Sarcocystis* species (Černá, 1984; Svobodová, 1996; Olias et al., 2011) and also intermediate hosts of *S. nontenella* and *S. otus* (Levine, Tadros, 1980; Krone et al., 2000). Recently an interesting model of *Sarcocystis* life cycle was disclosed, i. e. it was established that the magpie (*Pica pica*) serves as a definitive host of *S. ovalis* from moose (Gjerde, Dahlgren, 2010).

**Investigation of *Sarcocystis* species found in birds in Lithuania.** 97 poultry (76 hens and 21 turkeys) were investigated for the presence of *Sarcocystis* sarcocysts. Cysts were found only in the muscles of one cock, and according to morphological characteristics these sarcocysts were attributed to *S. horvathi*. During the period of 1986–1997 in Lithuania and in Russia (South Curonian Spit) out of 375 wild birds of 45 species belonging to Passeriformes, Piciformes, Charadriiformes, Columbiformes, Strigiformes orders examined, sarcocysts were detected only in three passerine birds, i. e. in the European robin (*Erithacus rubecula*), pied flycatcher (*Ficedula hypoleuca*) and fieldfare (*Turdus pilaris*) (Griekienienė, Iezhova, 1998).

Later in Lithuania *Sarcocystis* were most intensively studied in bird order Anseriformes. In 1997–1998, out of 25 birds analyzed, sarcocysts were found in five white-fronted geese (*Anser albifrons*), in four mallards and in one common shelduck (*Tadorna tadorna*) (Kutkienė et al., 1998). 29.2% (100 infected out of 342 examined) *Sarcocystis* infection prevalence was reported during a broader research in bird order Anseriformes (Kutkienė, Sruoga, 2004). Sarcocysts were detected in the muscles of the white-fronted goose, bean goose (*Anser fabalis*), lesser white-fronted goose (*Anser erythropus*), mallard, gadwall (*Anas strepera*), garganey (*Anas querquedula*), common teal (*Anas crecca*), common scoter (*Melanitta nigra*), velvet scoter (*Melanitta fusca*), shelduck, tufted duck (*Aythya fuligula*), long-tailed duck (*Clangula hyemalis*), common goldeneye (*Bucephala clangula*), common merganser (*Mergus merganser*) and common eider (*Somateria mollissima*). The highest prevalence of infection (65.8%) was determined in the white-fronted goose, while the highest intensity of infection was established in the white-fronted goose and long-tailed duck, 66 and 53 cysts in 28 muscle sections, respectively. By light microscopy, four types of microcysts were distinguished, whereas macrocysts were found only in neck muscles of one mallard.

On the basis of the results of the cyst wall ultrastructure and DNA analysis, using 18S rRNA gene, 28S rRNA gene and ITS-1 region genetic markers, three new *Sarcocystis* species from Anseriformes were described: *S. wobeseri* parasitizing the barnacle goose (*Branta leucopsis*) and mallard, *S. anasi* from the mallard, and *S. albifronsi* from

the white-fronted goose (Butkauskas et al., 2007; Kutkienė et al., 2006, 2008, 2010, 2012). Experimentally it was showed that the arctic fox (*Alopex lagopus*) is one of definitive hosts of *S. albifronsi* (Kutkienė et al., 2006). According to the ultrastructure of cysts and DNA analysis results, macrocysts isolated from a naturally infected mallard duck from Lithuania were identified as *S. rileyi* (Fig. 3) (Kutkienė et al., 2011). This was the first well-documented case of *S. rileyi* infection in Europe. As the range of the striped skunk is exclusively North America, it is unclear what animal contributes to the distribution of *S. rileyi* in Europe. In order to answer the question about the prevalence of *S. rileyi* in Lithuania and Europe, much more detailed investigations are necessary.

Macrocysts are predominantly found in the breast muscles, but so far mainly only leg and neck muscles have been analyzed. Therefore, examination of the breast muscles of ducks in different regions of the continent is advisable.

Kutkienė et al. (2009) investigated 67 birds of the family Corvidae in Lithuania and found microcysts in 16 individuals (23.9%): 14 hooded crows (*Corvus cornix*), one jay (*Garrulus glandarius*) and one rook (*Corvus frugilegus*). The highest prevalence of infection (35.9%; 14 infected out of 39 investigated) was accessed in the hooded crow. In the rook and the jay only sarcocysts with a smooth cyst wall were detected, whereas sarcocysts in hooded crows had radial spines or a smooth outer surface of the cyst wall. Combining morphological and DNA sequences data, sarcocysts having a striated cyst wall isolated from hooded crows were described as a new species of *Sarcocystis* – *S. cornixi*.

The cysts of *Sarcocystis* were found in leg muscles of two out of 18 wood pigeons (*Columba palumbus*) hunted in Lithuania and were identified as *S. columbae* (Prakas et al., 2011a). Also, in four out of 11 investigated herring gulls (*Larus argentatus*) *Sarcocystis* sp. microcysts were observed. *Sarcocystis* sp. from herring gulls had no significant morphological and genetic differences compared with *S. wobeseri*. Since parasitism of one *Sarcocystis* species in two or more species of intermediate hosts of different order is a very rare phenomenon, without evidence of cross-transmission experiments the sarcocysts extracted from herring gulls were named as *S. wobeseri*-like (Prakas et al., 2011b).





Fig. 3. Macrocysts of *S. rileyi* in the breast muscles of the mallard (Kutkienė et al., 2011)

***Sarcocystis* in reptiles.** Approximately 20 mild pathogenic *Sarcocystis* species forming sarcocysts in muscle tissues of reptiles are known (Odening, 1998). Also, about 25 *Sarcocystis* species are transmitted via reptiles. Small rodents: rats, mice, voles, hamsters and etc., are mainly intermediate hosts of these *Sarcocystis* spp. (Matuschka, 1987). It is worth noting that studies of reptile *Sarcocystis* have not been carried out in Lithuania so far.

#### Relevance of *Sarcocystis* studies

In summary, the representatives of the genus *Sarcocystis* are important parasites of domestic and wild animals. Despite the fact that *Sarcocystis* species have been broadly studied, the species composition, prevalence of infection and pathogenicity of *Sarcocystis* in many wild animal species is not accurately known. Also, further studies in chemoprophylaxis and chemotherapy of *Sarcocystis* are required. From the theoretical viewpoint, the knowledge of *Sarcocystis* species, intermediate host specificity, parasite-host evolution, ecological parameters of *Sarcocystis* infection are relevant.

It should be noted an important contribution of Lithuanian scientists to the studies of *Sarcocystis* diversity and life cycle.

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## SARCOCYSTIS PARAZITAI IR JŲ TYRIMAI LIETUVOJE

### *S a n t r a u k a*

*Sarcocystis* genties atstovai – tai cistas sudarančių kokcidijų grupės vienaląsčiai parazitai, plačiai paplitę įvairiose žinduolių, paukščių ir roplių rūšyse. *Sarcocystis* genčiai šiuo metu priskiriama per 220 rūšių. Kai kurių *Sarcocystis* rūšys yra žmogaus, naminių ir laukinių gyvūnų patogenai. Žalingas *Sarcocystis* parazitų infekcijos poveikis daugiausia pasireiškia tarpiniuose šeimininkuose, o patogeniškumas priklauso nuo tam tikros *Sarcocystis* rūšies, užsikrėtimo intensyvumo ir parazito lokalizacijos šeimininko organizme. Lietuvoje plačiausiai tirtos *Sarcocystis* rūšys, formuojančios sarkocistas graužikuose, porakanopiuose ir paukščiuose. *Sarcocystis* parazitų ekologija ir įvairovė tirta tradiciniais morfologiniais, DNR analizės metodais, taip pat eksperimentų metu, kai organizmai buvo specialiai užkrečiami parazitais. Lietuvos mokslininkai aprašė penkias naujas mokslui *Sarcocystis* rūšis – *S. rodentifelis*, *S. wobeseri*, *S. cornixi*, *S. anasi*, *S. albifronsi*; pirmą kartą pateikė svarius *S. rileyi* infekcijos Europoje įrodymus; nustatė kai kurių *Sarcocystis* rūšių galutinius šeimininkus.

**Raktažodžiai:** *Sarcocystis*, patogeniškumas, įvairovė, tyrimai Lietuvoje