LABORATORY IDENTIFICATION OF *Sarcocystis* spp. IN BIOLOGICAL SAMPLES FROM PIGS SLAUGHTERED FOR COMMERCIAL AND HOUSEHOLD CONSUMPTION IN DOLJ COUNTY, ROMANIA

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Abstract. Through a series of sampling and examination of skeletal muscle samples collected from a total of 10,410 pigs, of which 10,314 were sacrificed normally in the three slaughterhouses in Dolj county between 2019-2024, and 96 were sacrifice for private consumption, it was found that in 17 samples (0.16%), the parasite *Sarcocystis* spp. was identified (order Sarcosporidia, family Sarcocystidae, Poche, 1913; subfamily Sarcocystinae, Poche, 1913; genus *Sarcocystis*, Lankaster, 1882). This is one of the dangerous parasites for both animals and humans. The presence of sarcocysts in the striated muscles, was highlighted through macroscopic and trichineloscopic examination by compression of the samples at LSVSA Dolj. In the absence of molecular biological analyses, a method we currently do not have access to, the identification of species was not possible.

Keywords: *Sarcocystis* spp., pigs, Dolj.

Rezumat. Identificarea de laborator a speciei *Sarcocystis* spp. în probe biologice prelevate de la porci sacrificați pentru consum comercial și casnic în județul Dolj, România. Printr-o serie de prelevări și examinări de probe de musculatură scheletică recoltate din totalul de 10410 porcine din care 10314 au fost sacrificate normal în cele trei abatoare din județul Dolj în perioada 2019-2024, iar 96 au fost sacrificate pentru consum propriu, s-a constatat că în 17 probe (0.16%) am identificat parazitul *Sarcocystis*spp. (ord.Sarcosporidia, fam. Sarcocystidae, Poche, 1913; subfam. Sarcocystinae, Poche, 1913; genul *Sarcocystis*, Lankaster, 1882), acesta fiind unul dintre paraziții periculoși atât la animale, cât și la om. Prezența sarcochiștilor la nivelul musculaturii striate a fost evidențiată prin examinarea macroscopică și trichineloscopică prin compresie a probelor în LSVSA Dolj. În absența unor analize biologice moleculare, metodă de care nu dispunem în prezent, identificarea speciei nu a fost posibilă.

Cuvinte cheie: *Sarcocystis* spp., porci, Dolj.

INTRODUCTION

Zoonoses still remain a current problem worldwide. Sarcocystosis, also called sarcosporidiosis, as a disease, is a parasitic disease one, common to humans and several species of domestic and wild animals. The *Sarcocystis* spp. parasite was discovered in 1843, in Switzerland for the first time in the muscle tissue of the house mouse by Miescher F., and in Romania in 1959 the first data on sarcocystosis in deer were reported (RIMAILA- ȘUTEU 1975, 1997; PARNANEN & NIKANDER, 1980; JENSEN et al., 1986; ȘUTEU & MIRCEAN, 1996; FISCHER & ODENING, 1998; ȘUTEU & COZMA, 1998).

In humans, the muscular sarcocystosis in our country was diagnosed (Panaitescu, Gh. Cristea, V. Jula and Eugenia Cristea for the first time in 1978. For a long time, the biological cycle was unknown. At first, it was considered non-pathogenic and with its description in 1972, it began to be studied in more depth in domestic and wild animals in several countries. Studies conducted on farm animals by Ghila I. et al. (1985) mention that cattle were infested in 0.17 – 1.0% of cases, sheep – 0.9 – 5.1% and pigs in 0.2 – 0.9% of cases, in Bihor County (Romania).

In 1996, O. Rotaru found that microscopic examination of muscle tissue fragments from 800 cattle, sheep, goats and pigs that are intended for human consumption, revealed a very high incidence of 70-96% of parasitism with intracellular schizogonic forms (KALYAKIN & ZASUKHIN, 1975; IEPURE & RUSU 1985; DUBEY et al., 1992; BRIGGS et al., 1993; DUBEY & BWANGAMOI, 1994; GJERDE, 2013; YE et al., 2018).

Sarcocystis spp. are intracellular protozoan parasites belonging to the phylum Apicomplexa, with a heteroxenous life cycle involving carnivores as definitive hosts and herbivores or omnivores as intermediate hosts. In swine, infections are most commonly caused by *Sarcocystis miescheriana*, a species transmitted through ingestion of sporocysts excreted in the feces of carnivores, particularly canids (DUBEY et al., 1989). Once ingested, the parasites undergo asexual reproduction in the vascular endothelium, followed by encystment in skeletal muscles, including the diaphragm, tongue, and intercostal muscles.

Although *Sarcocystis* infections in pigs are often asymptomatic, high parasite loads may cause clinical signs such as fever, myositis, or even sudden death in severe cases. More frequently, the disease remains subclinical, with parasitic cysts being discovered incidentally during post-mortem meat inspections. The identification of sarcocysts is thus of particular importance in the context of food safety and zoonotic risk, especially considering that some *Sarcocystis* species are potentially transmissible to humans through the consumption of undercooked or raw meat (CASTRO-FORERO et al., 2022).

In many rural areas, pigs are slaughtered outside of official veterinary control systems, often for household consumption. This practice significantly increases the risk of undiagnosed parasitic infections entering the food chain. Macroscopic examination of muscle tissue during home slaughter, followed by confirmatory methods such as

compression trichinoscopy, remains a practical approach for preliminary parasitological screening under such conditions (EFSA, 2013).

Sarcocystis spp. presents an evolutionary cycle that includes as definitive hosts (intestinal form) in general, carnivores and humans, and as intermediate hosts (muscular form) a wide variety of mammals, birds, reptiles, fish and humans. The ingested parasites cross the intestinal walls, enter the blood and reach the muscle tissue where they form cysts, especially in: the masseter muscles, the chest, shoulder, trunk and thigh muscles. As a rule, cysts, located in the muscle tissue of animals, are transmitted to humans through insufficiently cooked or thermally prepared meat, reaching the human intestine where they release merozoites, which penetrate the intestinal epithelium and cause digestive disorders of varying severity (OLTEANU, 1999).

The present study aims to evaluate the occurrence and diagnostic detectability of *Sarcocystis* spp. in pigs slaughtered for personal consumption by employing both macroscopic inspection and trichinoscopic examination of diaphragmatic muscle samples.

MATERIAL AND METHODS

Muscle samples were collected from the diaphragmatic pillar muscles of 96 domestic pigs (*Sus scrofa domesticus*) slaughtered for personal consumption in rural households. The animals were not subjected to prior veterinary inspection, and sampling was performed post-mortem during routine househ. This work describes the presence and morphological characteristics of *Sarcocystis* spp. identified in skeletal muscle samples collected from a total of 10,410 pigs. Of these, 10,314 animals were slaughtered under standard conditions in the three officially approved slaughterhouses of Dolj County between 2019 and 2024, while 96 pigs were slaughtered for personal consumption. Particular attention is given to the parasitic burden observed and the potential complications associated with high tissue invasiveness, as well as to the zoonotic impact on the definitive host – humans – especially in cases of consumption of undercooked or raw infected meat.old processing. This study was conducted on a total of 5,376 biological samples (skeletal muscle tissues) suspected of sarcocystosis, collected from 96 pigs slaughtered for personal consumption. Additionally, a set of 560 biological samples was randomly selected from pigs slaughtered in authorized slaughterhouses and subjected to both macroscopic inspection and compression trichinoscopy; all examined samples in this group tested negative for *Sarcocystis* spp. infection. It is noteworthy that all muscle samples were transported to the laboratory in refrigerated containers, maintained at a constant temperature of 4°C to preserve tissue integrity. According to the specialized literature, optimal preservation methods for molecular identification of *Sarcocystis* spp. include freezing at –20°C, refrigeration at 4°C, or fixation in 90% ethanol.

The parasite and its intramuscular sarcocysts were identified primarily through trichinoscopic examination by compression, although macroscopic detection was possible in cases of severe and chronic infestations. The method employed followed the protocol outlined in the 'Standard Operational Procedure for the Identification of Parasite Species of the Genus *Sarcocystis* spp. in Animals by Trichinoscopy' (ȘUTEU et al., 1997).

For trichinoscopic analysis, certain muscle samples were preserved at 4°C during transport to the laboratory to ensure the integrity of tissue and cysts. The examination focused on striated muscle tissue, using a protocol analogous to that applied for the detection of *Trichinella* spp., which involves sectioning 28 oat-grain-sized pieces from each muscle along the direction of the fibers. These sections were positioned in fields numbered 1 to 28 on the lower plate of the trichinoscope compressor. Each was then compressed using the upper plate.

The trichinoscopic examination was conducted under 40× magnification for routine screening, with 80× magnification employed for enhanced detail and confirmation of morphological structures. The assessment consistently began with field 1 and proceeded sequentially through field 28. In each field, both the muscle tissue and the surrounding fluid were meticulously examined for the presence of sarcocysts (Figs. 1a, b).

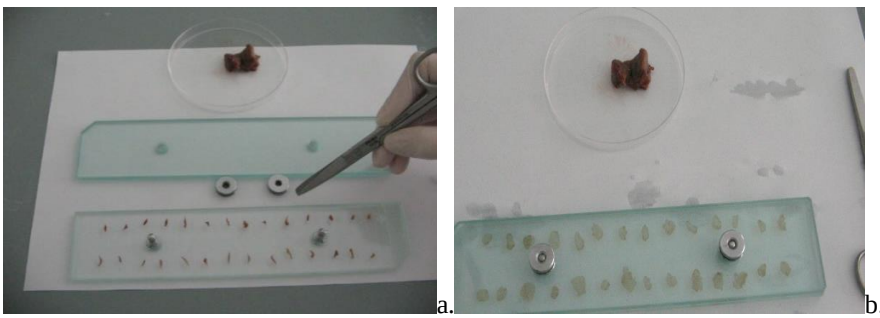
a. b.

Fig 1 (a, b). Trichineloscopic exam – spread (a), compression (b) (photo: Tîmburescu & Borontea, 2024).

RESULTS AND DISCUSSIONS

The primary objective of this study was the macroscopic identification and microscopic characterization of sarcocysts located in striated muscle tissue, using trichinoscopic examination by compression. Sarcocystosis is a chronic parasitic disease that primarily affects the muscular system of intermediate hosts, leading to the formation of distinct cysts within the intramuscular connective tissue. Despite its zoonotic and epizootological significance, sarcocystosis remains an underdiagnosed and undervalued parasitosis in Romania.

The biological cycle of *Sarcocystis* spp. is obligately heteroxenous, involving a strict predator–prey relationship between the two hosts. The asexual developmental stages occur exclusively in the intermediate host—typically a prey species—while the sexual stages are restricted to the definitive host, which is usually a carnivorous animal. In the present study, pigs originating from farms and rural households serve as intermediate hosts, acquiring infection through ingestion of sporocysts present in contaminated feed, water, or the environment.

Muscle samples were collected bilaterally from the diaphragmatic pillar muscles of 96 pigs slaughtered for personal consumption. Of these, 17 samples tested positive for *Sarcocystis* spp., confirmed through compression trichinoscopy, with several cases also exhibiting visible macroscopic lesions. Trichinoscopic examination was performed systematically, and in instances where suspicious structures were identified, the fields were further investigated using a higher magnification objective (100×) for enhanced resolution. In cases of diagnostic uncertainty, additional tissue samples were collected and re-examined through repeated compression until definitive results were obtained (Fig. 2).

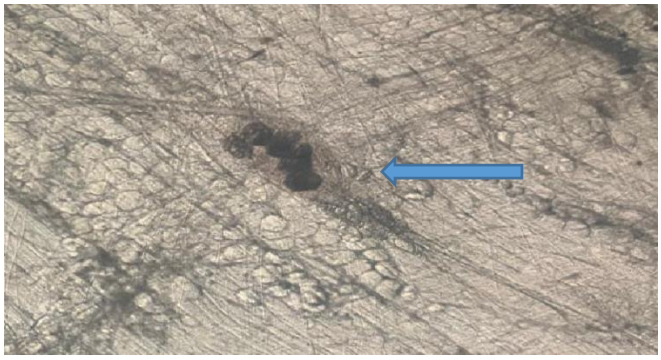

Figure 2. *Sarcocystis* spp. – Cysts, visualization of pig muscle fiber with trichinoscope by compression, 80X objective (photo: Tîmburescu & Borontea, 2024).

The diagnosis of sarcocystosis caused by *Sarcocystis* spp. in intermediate hosts can occasionally be made during slaughter; however, it is not considered a reliable diagnostic method unless macroscopic evidence is present. Specifically, the identification of fusiform, yellowish-white, granular cysts containing rounded corpuscles within and located between muscle fibers is required for a presumptive diagnosis (Fig. 3).

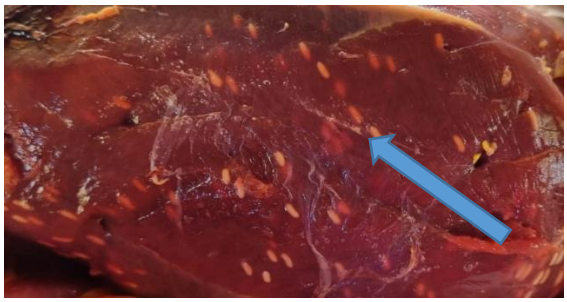

Figure 3. *Sarcocystis* spp.– Cysts, macroscopic view, muscletissue in a pig slaughtered for own consumption (photo: Borontea & Tîmburescu, 2024).

In the 17 muscle samples from pigs slaughtered for personal consumption, sarcocysts measuring between 0.5 and 1.5 mm were identified, visible to the naked eye an aspect that facilitates suspicion of disease during sanitary-veterinary meat inspection. It is important to note that species-level identification of *Sarcocystis* can only be achieved through molecular biology techniques, which were not available for this study. However, it is well established that the species with zoonotic potential are *Sarcocystis suihominis* and *Sarcocystis bovihominis* (OLTEANU, 1999). In humans, intestinal sarcocystosis is acquired through the consumption of raw or undercooked beef or pork containing mature sarcocysts. In animals, infection occurs via ingestion of sporocysts excreted in the feces of definitive hosts, contaminating pastures, feed, or water sources. Given the public health relevance and the potential impact of sarcocystosis, preventive measures are essential. These include avoiding the consumption of raw or insufficiently heat-treated meat (cooked below

60°C), or freezing meat at –20°C for at least 3 days prior to consumption. Non-compliance with these recommendations may lead to toxic syndromes in humans, caused by sarcocystin, with clinical manifestations such as anxiety, nausea, gastrointestinal pain, and edema, typically lasting between 24 and 36 hours.

The detection of sarcocysts through both macroscopic observation and trichinoscopic confirmation underscores the importance of thorough meat inspection, even in animals slaughtered outside of authorized facilities. The identification of lesions during gross examination can serve as an early indicator for targeted parasitological analysis. The size range and appearance of the cysts are consistent with previously documented infections in swine, most frequently attributed to *Sarcocystis miescheriana* (DUBEY et al., 1989; CASTRO-FORERO et al., 2022).

CONCLUSIONS

Muscle samples from both diaphragmatic pillar muscles from the 96 pigs sacrifice for own consumption and 17 samples were confirmed macroscopically, which is an advantage in the sanitary-veterinary examination of meat, leading to suspicion of disease. The trichinelloscopic examination by compression followed with confirmation of the presence of sarcocysts that vary in size between 0.5 and 1.5 mm. In this context, the monitoring of sarcocystosis in domestic (cattle, sheep, dogs, cats) and wild (bear, wild boar) animals from different habitats has a bioecological, medical and veterinary importance in preventing the transmission of sarcocysts to humans and other mammals involved in the biological cycles of parasites, which is why measures to reduce the level of infestation in wild animals are required.

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