INTRAMUSCULAR *SARCOCYSTIS* CYSTS DETECTION IN ANIMALS: A REVIEW ARTICLE

FLAVIU OTNIEL CIOBOTĂ, MARIANA IONIŢĂ, IOAN LIVIU MITREA

University of Agronomical Sciences and Veterinary Medicine of Bucharest,
Faculty of Veterinary Medicine, Bucharest, Romania
flaviuciobota@yahoo.ro

Abstract:*Sarcosporidiosis is a cosmopolitan zoonotic parasitic disease caused by small intracellular apicomplexan protozoa of the genus Sarcosystis. This parasite has a heteroxenous life cycle, involving: a definitive host, where Sarcocysts spp. develops in intestine, infesting the enterocytes, and an intermediate host, in which invades the muscle cells in order to form the typical sarcocysts in tissue. Sarcocystis species parasitize almost all vertebrates and are widely distributed around the globe. So far, 189 species of Sarcocystis have been identified; however, for the both definitive and intermediate hosts are known only 46% of the species. The purpose of this literature review is to present reported data on the prevalence of intramuscular Sarcocystis cysts in animals. Data from various studies suggest different variations depending on the species and the area where research was performed, such as in cattle (24–80%), swine (0.80-43%), goats (52-79%), and sheep (18-100%). The field research refers to the degree of intensity and extent of the skeletal muscles microsarcocysts in the following anatomical regions: the intercostal muscles, the diaphragm, tongue muscles, and dorsal and hamstring muscles. From all the Sarcocystis species found in animals, only two of them (S. hominis and S. suihominis) have zoonotic potential, the man can be intermediate host for their species and also definitive host for species mentioned. Considering the fact that the sarcosporidiosis is high-impact diseases among animals, data discussed in this paper emphasize the presence of intramuscularly sarcocysts, as well as the zoonotic risk in several regions of the world in the last decade.*

Key words: *Sarcocystis spp.,* animals, intramuscular cysts, epidemiology

INTRODUCTION

Sarcocystis genus is a specific group of protozoa that infects intracellularly, and represents the end stage of the parasite asexual life, found in the tissue of its host (Mitrea, 2011). This parasite has a heteroxenous life cycle, involving: a definitive host, where *Sarcocysts* spp. develops in intestine, infesting the enterocytes, and an intermediate host, in which invades the muscle cells in order to form the typical sarcocysts in tissue (Dubey et al., 1989).

Sarcocystis spp. was reported in 1843 by Miescher, describing whitish cysts, threadlike, in striated muscles at the house mouse, lacking a scientific name, and after 20 years, the parasite was named as "Miescher's tubules" (Fayer, 2004). Over time, scientists have debated whether *Sarcocystis* species are fungi or protozoa. There have been theories that *Sarcocystis* were fungi, because only sarcocyst stage was known at the time, and when sarcocysts and their contents were placed in different culture media, mycelia and hyphae were sometimes found several days later. After the first reporting of *Sarcocystis*, the needle or crescent-shaped bodies (bradyzoites) in sarcocysts were studied by electron microscopy, and showed similarity with other aplicomplexan protozoa such as *Toxoplasma* and *Eimeria* (Fayer, 2004).

Although it is a parasitosis with discrete clinical events, the surveys show a special medical and veterinary significance. From all the *Sarcocystis* species found in animals, only two of them (*S. hominis*, from cattle, and *S. suihominis*, from swine) have zoonotic potential, the man can be intermediate host for their species and also definitive host for species mentioned (Dubey, et al., 1989).

The purpose of this literature review is to present reported data on the prevalence of intramuscular *Sarcocystis* cysts in animals.

Intramuscular *Sarcocystis* in animals

Sarcocystis species parasitize almost all vertebrates and are widely distributed around the globe. So far, 189 species of *Sarcocystis* have been identified; however, for the both definitive and intermediate hosts are known only 46% of the species (Odening, 1998), for example, in animals of the *Bovidae* family (domesticated *Bovidae*) have been identified 143 species of *Sarcocystis* (Heller et al., 2013).

Some species of *Sarcocystis* are always microscopic (e.g. *S. cruzi*), while other species becomes macroscopic (e.g. *S. gigantea* and *S. muris*). Common forms of sarcocysts are filamentous, elongated or globular. Macroscopic sarcocysts are almost always in skeletal muscle or esophageal muscles (Dubey et al., 1989). Parasites identification is based on observation of sarcocysts in muscle tissue. In the muscle preparations, sarcocysts size up to 1 cm long (Greiner et al., 1989).

As a result of the consumption of meat containing sarcocysts by the definitive host, the sexual phase of the life cycle of the parasite occurs in the host's intestinal wall and ends with the release of sporocysts with the elimination of faeces into the environment. Following ingestion of oocysts by the intermediate host, the parasites enter the asexual reproduction phase, which through four generations of merozoites leads to the formation of sarcocysts inside the muscle cells of the host (cardiac, striated, or smooth muscle) (Domenis et al., 2011). The field research refers to the degree of intensity and extent of the skeletal muscles microsarcocysts in the following anatomical regions: the intercostal muscles, the diaphragm, tongue muscles, and dorsal and hamstring muscles (Tăbăran et al., 2013).

Data from various studies suggest different variations depending on the species and the area where research was performed, such as in cattle (24–80%), swine (0.80-43%), goats (52-79%), and sheep (18-100%).

***Sarcocystis* infection in cattle**

Heydorn et al. (1975) showed that conclusively there were three species of *Sarcocystis* in cattle and proposed new names for them: *S. bovicanis* having definitive host the canids, *S. bovifelis* having definitive host the felines, and *S. bovihominis* having definitive hosts the both primates and humans (Heydorn et al., 1975). The new names of these species have been rejected by the International Code of Zoological Nomenclature in favor of the existing designations: *S. cruzi, S. hirsuta* and *S. hominis* (Frenkel et al., 1979).

Exists a considerable confusion as to *Sarcocystis* species in buffalo (*Bubalus bubalis*), and in cattle (*Bos taurus*). This issue is of current interest, because some species such as *S. hominis* in cattle prove to be zoonotic, but the same cannot be said for the species of buffalo (Jehle et al., 2009; Yang et al., 2001a,b).

Although the *Sarcocystis* spp. infections were reported after more than a century in Hungary, the actual significance of infection in cattle with *Sarcocystis* is not known in the country, neither throughout Central and Eastern Europe. In 2014, in a study conducted by Hornok et al., there have been examined 151 cattle and 15 buffalos. In total, 100 of the 151 cattle (66.22%) were positive diagnosed for *Sarcocystis* infestation by PCR, while at buffalos has not been find any positive sample (Hornok et al., 2015).

A study conducted in north-western Italy, Domenis et al. (2011) identified the presence of *Sarcocystis* cysts in cattle by rapid histological examination of the diaphragm and cardiac muscle, and esophagus, and by conventional electron microscopy in combination with molecular

techniques. Through histological screening, 78.1% (300/384) of the animals tested were found to sarcocysts in at least one organ. The most frequent affected anatomical structure was esophagus (n=279), then the diaphragm muscle (n=228), and cardiac muscle (n=225). Through molecular techniques it was identified all 3 *Sarcocystis* species that are found in cattle (*S. cruzi, S. hominis* and *S. hirsuta*). The prevalence of these species detected in samples population was : 74.2% *S. cruzi* (n=285), 42.7% *S. hominis* (n=164), 18.5% *S. hominis*-like (n=71) and 1.8% *S. hirsuta* (n=7) (Domenis et al., 2011).

In Argentina, Moré et al. performed a study on 90 beef cattles, to diagnose infection with *Sarcocystis* sp. and to detect co-infections with *Neospora caninum* and/or *Toxoplasma gondii*, collecting the following sections: 50g of diaphragm muscle, 100g of myocardium (left ventricle), and 10 cm of esophagus, from each animal. After microscopic examination, this sarcocysts was found 100% in the myocardium, 71% in esophagum, and 28% in the diaphragm muscles. The characteristics of *Sarcocystis* cysts detected corresponded with *S. cruzi*: that shows thin walls (<1 µm thick), and villar protrusions had lengths between 6-13µm (Moré et al., 2008).

Sarcocystis infection in swine

Of the three of *Sarcocystis* species in pig (*S. miescheriana, S. porcifelis* and *S. suihomini*), only *S. suihominis* is important in public health. The vast majority infections in pigs are asymptomatic (Avapal et al., 2004).

In a study performed in Romania (Transylvania area) by Tăbăran et al. (2013) have been examined 150 of meat samples, of which 120 from domestic swine (diaphragmatic pillars) and 30 from wild boars (muscle tissue). The samples were examined for infection with *Trichinella* and *Sarcocystis*. Following the examination by classical trichinelloscopy were found a large numbers of infection with *Sarcocystis* spp cysts. Of the 150 meat samples, 97 (64%) were positive for *Sarcocystis*, of which 67 (56%) in domestic swine and 30 (100%) in wild boar. Comparing the statistics for these two species, it can be stated that the infection is higher in wild boar meat than it is in pig (Tăbăran et al., 2013).

In 1997, Saito et al. identified for the first time in Japan in pigs *S. suihominis*. In their study, cardiac and diaphragm muscle from the 600 breeding pigs reared in the same piggery were examined. Of the 600 pigs examined, 5 animals (0.83%) were positive for *Sarcocystis* cysts. Fresh cysts measured 1.080-2.040 x 106-107 µm length and 4-6 µm thickness (Saito et al., 1998).

Sarcocystis infection in goat

So far three species of *Sarcocystis* spp. have been identified in domestic goats: *Sarcocystis capracanis, S. hircicanis* and *S. caprafelis*. Also, *S. capracanis* and *S. hircicanis* produce microscopic sarcocysts (microcysts), while *S. caprafelis* produce macroscopic cysts (macrocysts). *S. capracanis* is the most pathogenic in goats, causing fever, weight loss, anorexia, tremors, irritability, abortion and death (Dubey et al., 1989).

In the study of Shekarforoush et al. in Iran, were examined 169 slaughtered goats, randomly selected. The animals were inspected for the presence of macroscopic sarcocysts, examining the tongue, heart, esophagous and various skeletal muscles, such as diaphragm, abdominal and intercostal muscles from each animal. 28 of the 169 goats (16.6%) were diagnosed as infected with macroscopic sarcocysts. Of the 169 goats, 168 were diagnosed as positive for *Sarcocystis* species by smear method, and all 169 samples were positive by digestion method. The sensitivity of the method spreading (smear method) compared to the digestion method in the

infection diagnosis was 94% in the heart, 92.3% in esophagus, 91% in tongue, and 84% in diaphragm (Shekarforoush et al., 2005).

***Sarcocystis* infection in sheep**

Four *Sarcocystis* species were reported in sheep: *S. gigantea, S. arieticanis, S. medusiforms*, and *S. tenella*, the later one being the most pathogenic in sheep (Heckeroth and Tenter, 1999; Dubey et al., 1989 a, c). Sheep can be infected by ingesting contaminated water or fodder with sporocysts. Clinical signs usually are reserved, but when are present include anorexia, fever, weight loss, anemia and even abortion (Silva et al., 2009).

The prevalence of *S. tenella* is variable throughout the world. In Asia is reported the highest prevalence: 33.9% in Iran (Daryani et al., 2006), 47.3% in Turkey (Őzkyhan et al., 2007), 93% in Ethiopia (Woldemeskel and Gebreab, 1996), 96.9% in Mongolia (Fukuyo et al., 2002).

In the study of epidemiology of sarcocystosis at sheep from Romania, conducted by Titilincu et al. (2008), were examined 48 sheep, with aged between 2-3 years. Of the 48 samples, 44 (91.7%) were found to be positive with *Sarcocystis* spp. The macroscopic examination were identified macrocysts only into the esophagus at 26.3% (10/38) examined carcasses. Microcysts were identified 81.6% (31/38) into esophagus, 79.9% (35/48) in myocardium, and 68.8% (33/48) in diaphragm muscles (Titilincu et al., 2008).

The increased prevalence is associated with the free access of dogs (definitive host) in sheep flocks, or arthropod that acts as mechanic vectors for sporocysts dog faeces excreted (Silva et al., 2009).

Heart, diaphragm and skeletal muscles are the organs most often parasitized by *Sarcocystis* spp. in the intermediate host (Buxton, 1998; Daryani et al., 2006). In a study conducted by Silva et al. (2009), were detected co-infection with *S. tenella* and *T. gondii* in the hart and diaphragm muscles, the animals not showing clinical signs (Silva et al., 2009).

Several studies in human population have been carried out for determination of human sarcocystosis. Prevalence data for *Sarcocystis* infection reflect mainly case reports and findings by physicians, public health workers and scientists with specific interests. Consequently, many infections are not reported (Fayer, 2004).

Humans can be in the same time intermediate host for their species and definitive host for *S. bovihominis* and *S. suihominis*. Consumption of raw meat or insufficiently prepared from pigs or cattle infected with *S. hominis* and *S. suihominis* produce the human infection (Van Knapen et al., 1987).

The anatomic regions most infected with *Sarcocysts* spp. in humans are skeletal and cardiac muscles, but there were, also, found in the larynx, pharynx and esophagus (Lele et al., 1986).

In conclusion, data from these studies concerning the presence of sarcosporydiosis are relevant to human and animal health, because, the incidence of *Sarcocystis* spp. in animals and humans has an increased extensivity with of sanitary and socioeconomic implications (Domenis et al., 2011).

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