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Natural *Sarcocystis gigantea* infection in sheep from Southern Brazil

Infecção natural por *Sarcocystis gigantea* em ovinos no Rio Grande do Sul

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– NOTE –

ABSTRACT

Protozoal diseases caused by species of *Sarcocystis* can cause serious damage in sheep flocks, inducing decreased growth conversion rates and partial or complete loss of carcasses at the slaughterhouse. This article describes an outbreak of *Sarcocystis gigantea* infection in sheep slaughtered in a farm in Rio Grande do Sul State, Brazil. Between July and September 2013, three sheep showed multiple nodules in the esophagus that were microscopically characterized as encapsulated cysts filled with elongated, basophilic, nucleated structures morphologically consistent with *S. gigantea* bradyzoites. Diagnosis was made based on the epidemiological, macroscopic, and microscopic findings. This is the first report of this infection in sheep in Rio Grande do Sul and should be recognized by veterinarians, especially during meat inspection.

Key words: sarcocystosis in sheep, apicomplexa, pathology.

RESUMO

Doenças causadas por protozoários do gênero *Sarcocystis* podem causar sérios prejuízos em rebanhos de ovinos, por induzirem retardo no crescimento e condenação parcial ou total das carcaças em abatedouro. O presente trabalho descreve a infecção natural por *Sarcocystis gigantea* em ovinos abatidos em uma propriedade rural no Rio grande do Sul. Entre julho e setembro de 2013, três ovinos apresentaram múltiplos nódulos no esôfago, os quais, microscopicamente, corresponderam a cistos circundados por cápsula rugosa, repletos de estruturas alongadas, basofílicas e nucleadas, morfológicamente compatíveis com bradizoítos de *S. gigantea*. Os dados epidemiológicos unidos aos achados macroscópicos e microscópicos permitiram o diagnóstico conclusivo. Esse foi o primeiro relato dessa infecção no Rio Grande Sul e deve ser reconhecida por veterinários de campo e especialmente no momento da inspeção das carcaças no abatedouro.

Palavras-chave: sarcocistose em ovino, apicomplexa, patologia.

Lesions caused by protozoal diseases can cause economic losses due to decreased production rates or death of the most severely infected animals (ADRIANA et al., 2008). Sarcocystosis caused by species of *Sarcocystis* is responsible for the formation of cysts in different organs, which are often observed in the skeletal and cardiac muscle, and has a high prevalence in sheep, resulting in serious injury in different organ systems, abortion, and death (MARTÍNEZ-NAVALÓN et al., 2012).

Sarcocystis gigantea is a parasite with worldwide distribution responsible for development of macroscopic cysts in sheep (BEYAZIT et al., 2007; MARTÍNEZ-NAVALÓN et al., 2012; DEHAGHI et al., 2013). It belongs to the phylum Apicomplexa, family *Sarcocystidae*, and genus *Sarcocystis*. It is also referred to as *Sarcocystis ovifelis*, because cats are the definitive hosts, while sheep are the intermediate hosts. *S. gigantea* is responsible for forming cysts mainly in the tongue and esophageal muscles, and their presence in the muscles and viscera of carcasses in the slaughterhouse causes economic losses due to the partial or total loss of carcasses (MARTINEZ-NAVALON et al., 2012). This study reports the occurrence of natural infection by *Sarcocystis gigantea* in sheep in Rio Grande do Sul.

Between July and September 2013, visits were made to a farm in Santana do Livramento that had 300 adult Corriedale sheep and 250 Corriedale lambs destined for slaughter. According to the owner,

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the flock was raised in consortium with cattle and horses and was fed with native pasture with mineral supplementation. During evaluation, 12 sheep (4-6 years old) were slaughtered in the farm. Three sheep showed white nodules in the esophageal muscles that were collected in 10% formalin and sent for histopathological evaluation. Cats and dogs had free access to pastures and livestock; they were observed on site during the slaughter and ingested uncooked meat and viscera from slaughtered sheep.

Macroscopically, multiple esophageal nodules were observed during slaughter. They were characterized by white, round or oval cysts with 1.5x1.0x1.0cm, distributed randomly throughout esophageal muscles (Figure 1A). Cysts had a white

capsule and internal gelatinous translucent material. After previous evaluation, fragments were embedded in paraffin, sectioned in 3µm for histopathology slides, and stained with hematoxylin and eosin (Figure 1B). Microscopically, the cysts showed irregularly shaped, eosinophilic wall with placentiform protrusions, and adjacent connective tissue capsule. Random, eosinophilic projections supported the basophilic, 4µm elongated structures, which had evident nucleus and granular cytoplasm that was morphologically consistent with bradyzoites of *Sarcocystis gigantea* (Figure 1C and 1D).

Diagnosis of sarcocystosis by *Sarcocystis gigantea* was confirmed by epidemiologic, macroscopic, and microscopic findings (DUBEY

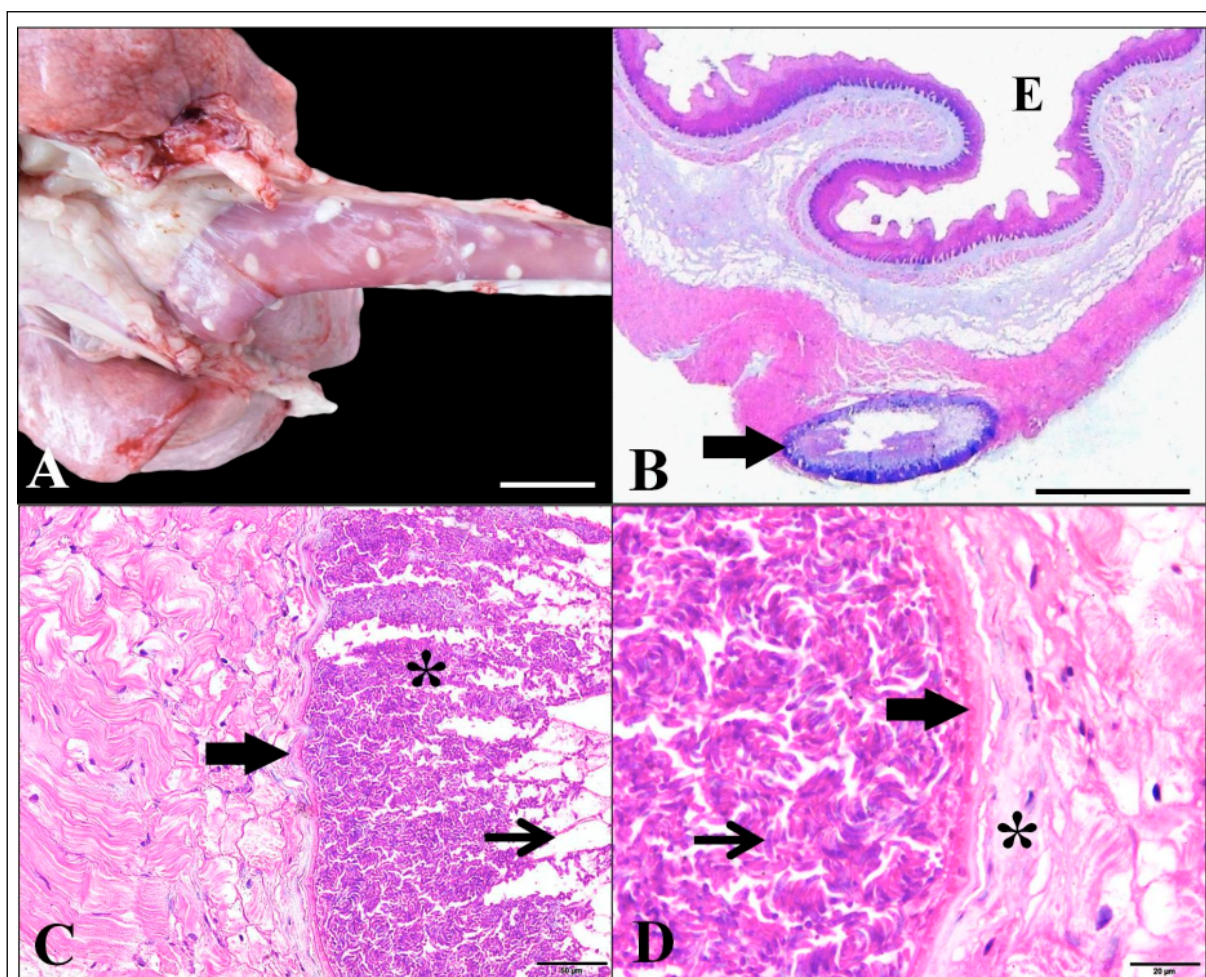


Figure 1 - *Sheep esophagus*: (A) Multiple oval and whitish cysts in the esophageal muscles. Bar = 2cm. (B) Cross section of esophagus with cysts of *Sarcocystis gigantea* in the muscular layer (thick arrow). *Esophageal lumen* (E). Hematoxylin and eosin. Bar = 1cm. (C) Striated skeletal muscle with cyst *S. gigantea*. Note that the wall of the cyst is irregular and surrounded by capsule (thick arrow) and inside are observed numerous basophilic structures (*) supported by thin septa from the capsule (thin arrow). Hematoxylin and eosin. Bar = 50µm. (D) Cyst of *S. gigantea* with eosinophilic internal capsule with placentiformes ripples (thick arrow). It is observed thin connective tissue capsule surrounding the cyst (*) and several elongated basophilic bradyzoites (thin arrow). Hematoxylin and eosin. Bar = 20µm.

et al., 1988; BEYAZIT et al., 2007; AL-HYALI et al., 2011). The main risk factors for infection are the contact of the definitive host with the agent and the supply of raw meat from sheep to cats, which contributes significantly to the propagation of sarcocystosis by *S. gigantea*, as well as other species of *Sarcocystis* sp. transmitted by canids (ADRIANA et al., 2008).

Sheep are infected with *S. gigantea* when they ingest oocysts or sporocysts that are eliminated with feces of cats (MCKENNA & CHARLESTON, 1988). Sporozoites originated meronts which, upon maturation, release merozoites that penetrate endothelial cells to originate secondary merozoites. These merozoites are released and can penetrate in muscle cells and will develop bradyzoites and cysts (MCKENNA, 1984). These cysts are similar to described in this note.

Most species of *Sarcocystis* that infect domestic animals are species-specific for the intermediate host (DUBEY et al., 1988). Sheep are intermediate hosts for four species of *Sarcocystis*: *S. gigantea* and *S. medusiformis*, which are transmitted by felids and are less pathogenic; and *S. tenella* and *S. arieticanis*, which are transmitted by canids and are more pathogenic, causing neurological disease and abortion (ADRIANA et al., 2008; DEHAGHI et al., 2013). The differentiation between *S. gigantea* and other species *Sarcocystis* was made based on the morphology of cysts (DUBEY et al., 1988; BEYAZIT et al., 2007). Cyst size varies according to the degree of maturation, and microscopically differentiation of each species and can be defined through the aspect of the wall. Shape and thickness of the wall can allow the definitive diagnosis (MARTINEZ-NAVALON et al., 2012; DEHAGHI et al., 2013).

Similar to the cases described in the current article, *S. gigantea* macrocyst showed irregular thick wall, with placitiform protrusions and a secondary wall of connective tissue (MUNDAY & OBENDORF, 1984). *S. medusiformis* also has thick wall, but induces the formation of smaller cysts. *S. medusiformis* geographical distribution is restricted to Australia, New Zealand, and Iran (FARHANG-PAJUH et al., 2014). Both *S. gigantea* and *S. medusiformis* are reported as non-pathogenic species, but can cause losses in the slaughterhouse and myositis when rupture of cysts occurs (AL-HYALI et al., 2011). This inflammatory lesion was not observed in the evaluated sheep.

Differently the previously mentioned species, *S. tenella* and *S. arieticanis* are pathogenic protozoa that cause microscopic cysts. *S. tenella*

has a thick wall and presence of villi without 2-4µm (BEYAZIT et al., 2007) and may cause nervous clinical signs, as well as abortion, decreased milk production, anemia, decreased weight gain and, in severe cases, death (SILVA et al., 2009). *S. arieticanis* showed cyst between 53-163µm diameter, and a thin wall with filiform projections with 6-12µm (DUBEY et al., 1988).

Several studies demonstrate a high prevalence of infections with *Sarcocystis* sp. in sheep (BEYAZIT et al., 2007; ADRIANA et al., 2008; MARTINEZ-NAVALON et al., 2012; BAHARI et al., 2014). In Brazil, studies of non-pathogenic species of *Sarcocystis* are uncommon and the occurrence of *S. gigantea* in sheep is underestimated in Rio Grande do Sul and Uruguay due to failure in the diagnosis. High prevalence of sarcocystosis by *S. gigantea* in sheep is reported in Iraq and mineralized cysts are observed in some cases (AL-HYALI et al., 2011). The high number of cases is a determining factor for reinfection of the definitive host and perpetuation of the life cycle of the parasite (DEHAGHI et al., 2013).

According to the farmer, young sheep were slaughtered on the property and cystic lesions were not observed. This may have occurred by the short exposure time of sheep to oocysts in the feces of the definitive host (cat) or the prolonged development of macroscopic cyst (MCKENNA, 1984). However, it has been described cysts in animals less than one year old, suggesting cyst growth less than one year (BEYAZIT et al., 2007). After the diagnosis, the owner was advised to not give carcasses remains and uncooked guts to cats in order to minimize contact between cats and sheep.

Although the lesions caused by *S. gigantea* are mild in most cases, in marked infections, the parasite can cause considerable losses in slaughterhouse (NAVALON-MARTINEZ et al., 2012). This report demonstrated the infection by *S. gigantea* occurs in sheep from Rio Grande do Sul. Lesions must be recognized by veterinarians, especially in the slaughterhouse. In addition, the diagnosis may indicate the infection by pathogenic protozoa of the phylum Apicomplexa in sheep, due to the similarities in protozoa life cycle.

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