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Short communication

Prevalence and pathologic changes due to *Sarcocystis* species in naturally infected sheep in Urmia city, Iran

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Summary

Sarcocystis species in animals and humans cause a zoonotic disease called Sarcocystosis. This study was aimed to investigate the morphology and pathology of Sarcocystisin naturally infected sheep. The carcasses of slaughtered sheep at Urmia slaughterhouse were inspected for evidence of infection with *Sarcocystis* macrocysts. Histopathological sections were prepared and stained routinely by Hematoxylin-Eosin (H&E) staining. A total of 1372 out of 4121 (33.3%) removed macrocysts were full of bradyzoites (54.3×223.66µm) ranged from 5-10 mm. Histologically, the reaction of the muscle tissue varied from degenerative to inflammatory around the macrocysts. The infected muscle demonstrated evidence of myocytolysis with infiltration of inflammatory cells in focal pockets, which were mainly comprised of lymphocytes and macrophages. The other findings were arterial wall hyperplasia, hyperplastic proliferation, and giant cell presence around the macrocysts. The results indicated that morphologic features of macrocysts of *Sarcocystis* in naturally infected sheep were informative and causing pathologic changes in muscle tissue.

Keywords: Sheep, Carcasses, Macroscopic *Sarcocystis*, Histopathology, Iran

Introduction

Sarcocystosis is one of the zoonotic diseases and coccidian parasitic infections, which causes macrocysts (Chena et al., 2010). The parasites are found in muscles of intermediate host and infect the gastrointestinal tract in carnivores as the definitive host. To date, 35 out of 93 identified species reported from 200 species of mammals (Dalimi et al., 2008). Four species of *Sarcocystis* have been reported in sheep, including *S. tenella*, *S.* arieticans, *S. gigantean*, *S. medusiformis* (Dubey, 2010). Humans can also be intermediate hosts for a variety of other

Sarcocystis species, *i.e.*, *S. suihominis* and *S. hominis*. In Iran, Sarcocystis infection in sheep was reported for the first time in 1972 (Chena et al., 2010; Dalimi et al., 2010). There were many report on Sarcocystis infection throughout the world and Iran (Oryan et al., 1996; Razmi and Rahbari, 2000; Shekarforoush and Alikhani, 2003; Bonyadian and Meshki, 2006; Dalimi et al., 2010).

The conventional method of distinguishing *Sarcocystis* species and combining these data with information on the life cycle are not suitable due to little morphological variation, high antigenic cross-reactivity, and time-consuming (Motamedi et al., 2010). However, laboratory studies on Sarcocystis infection like histopathology reported the first in the brain of sheep in Iran in 2006 (Bonyadian and Meshki, 2006). Thus, the present study was carried out to determine the macrocyst of *Sarcocystis* species using laboratory studies.

Materials and methods

The esophagus, diaphragm, and skeletal muscles in carcasses of sheep were thoroughly inspected for the presence of *Sarcocystis* macrocysts at Urmia industrial abattoir. The macrocysts were removed, excised from the tissues, and identified based on the morphologic and morphometric characteristics.

Twenty grams of pooled muscles were digested in 50mL of acid pepsin and incubated for 30min at 40˚C. The digested specimen was filtered through a fine-meshed sieve into a tube, centrifuged at 2000*g*×for 5min, and the sediment suspended in 0.5mL of distilled water. The suspension was then microscopically examined for the presence of *Sarcocystis* bradyzoites under the light microscope at 400× magnification (Arshad et al., 2007; Dalimi et al., 2008).

All infected tissue muscles with *Sarcocystis* macrocysts were fixed in 10% formalin and paraffin. The tissues were sectioned in 4-5μm thick pieces and stained with Hematoxylin and Eosin (H&E) staining and examined under a light microscope at 400×-1000× magnification (Dubey et al., 2000).

Results

The macrocysts of *Sarcocystis* occur as elongated cylindrical bodies, and milky-white colored cysts embedded in the muscular tissues with length ranged from <5 mm to >10 mm (Fig. 1). The inspected macrocysts were fat (29.3%, 1372/4121) in striated muscles of that were large enough to discriminate by the naked eye. The macrocysts were fully packed with banana- shaped bradyzoites averaging 5.43 × 22.36 μm (range: 3.16 to 7.38 × 18.77 to 27.69 μm) (Fig. 1). Based on this finding,

identified macrocysts maybe belonging to *Sarcocystis gigantea* (Fig. 1a-c).

Histopathological analysis of macrocysts of *Sarcocystis* revealed degenerative, myocytolysis, and inflammatory reaction and fibroblastic proliferation along with fibroblast-like and inflammatory cell infiltration, *i.e.*, lymphocytes and macrophages (Fig.2a-c). The vascular lesion included vascular proliferation and wall hyperplasia in the infected muscle (Fig.2-b).

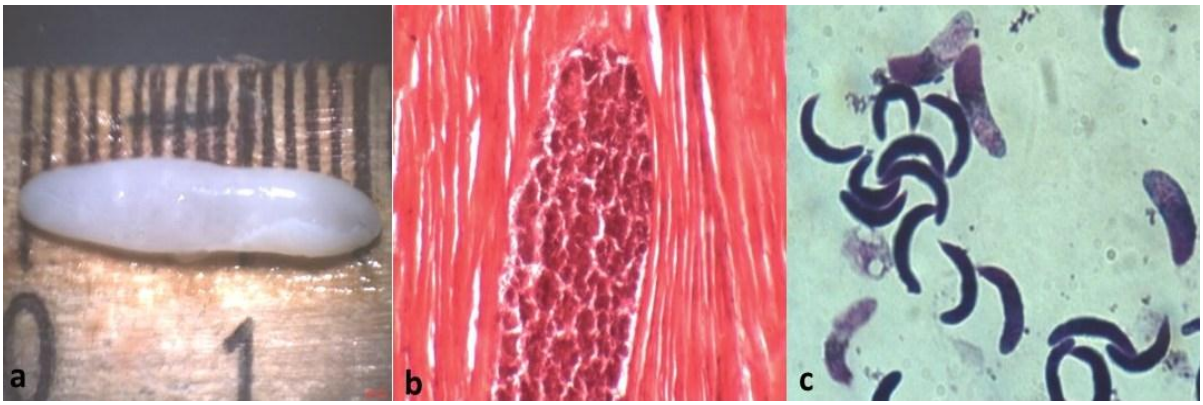

Fig. 1. A macrocyst of *Sarcocystis* in the diaphragm of naturally infected sheep **(a)**, the cyst initiated no tissue reaction **(b)**, bradyzoites **(c)** (H&E, 400×).

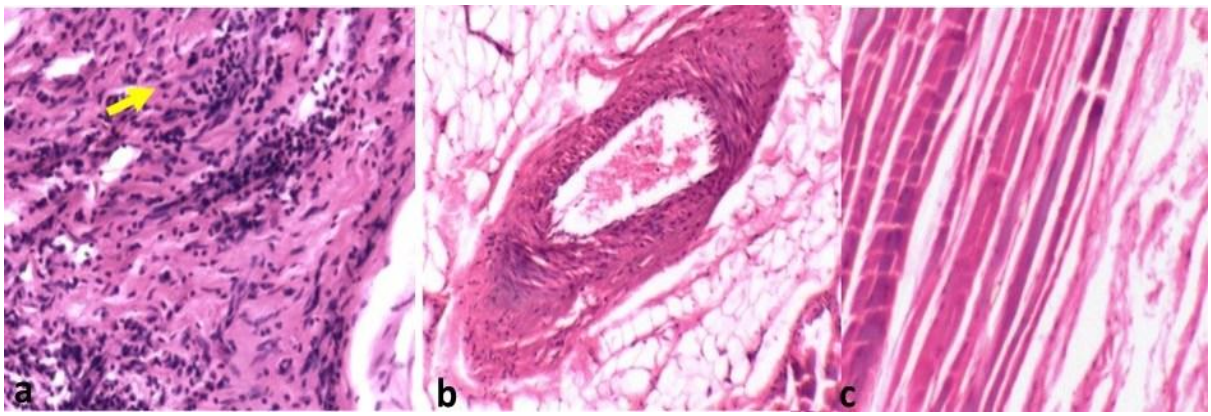

Fig. 2. Histologic sections of *Sarcocystis*: infected muscle showing evidence of myocytolysis **(a)**, arterial wall hyperplasia in the infected muscle **(b)**, part of a macrocyst along with inflammatory reaction, and fibroblastic proliferation **(c)**, macrophage (yellow arrowhead) (H&E, 400×).

Discussion

Sarcocystis is a ubiquitous protozoan and causes a mild infection in sheep but is important in meat inspection (Costa da Silva et al., 2009). In Iran, *S. gigantea* (synonyms: *S. ovifelis*) has spread worldwide, and its

oocysts are produced and excreted by cats and red foxes (Kojouri et al., 2011). Small ruminant infection with *S. gigantea* through close contact with cats and harboring oocysts in the pasture may be one of the main sources of high *Sarcocystis* infection in Iranian sheep (Dalimi et al., 2010). In most carcasses, infected animals with *Sarcocystis* macrocysts identified when doing meat inspection and observed between the muscle fibers (Yakhchali et al., 2010). In the present study, identified macrocysts as *S. gigantea* were in the form of elongated cylinders, which mainly reported from tissues of skeletal muscles of the diaphragm and esophagus. *Sarcocystis* macrocysts infection in sheep reports during the slaughterhouse inspections and laboratory studies (Razmi and Rahbari, 2000). During the meat inspection and chronic Sarcocystosis in sheep, *Sarcocystis* macrocysts were mostly present in the skeletal muscles of the esophagus and diaphragm (Oryan et al., 1996). In accordance with the current study, macroscopic lesion of *S. gigantea* in skeletal muscles was informing of strips of pale lines with petechia and ecchymosis. Fat atrophy around the heart and kidney, edema, focal necrosis in lymph nodes, and blooding wounds in a serous visceral level also observed as described by Beyer (2001).

Microscopically, in the current study, the tissue muscles infected with *S. gigantea* were in the form of inflammatory, degenerative, and myocytolysis around the macrocysts. There was no inflammatory cell infiltration, degenerative, and inflammatory lesions around the necrotic and degenerated microcysts in examined tissues. The influence of interstitial connective tissue caused the rupture of the muscle fibers. In this study, the focal accumulation of lymphocytes and macrophages, and vascular proliferation around the arterial vessel wall proposed. Dubey and Rommel (1992) noted that the lesions were microscopically in the form of hypertrophy of vascular endothelial cells, which were often along with infiltration of lymphocytes, neutrophils, macrophages, giant cells, few eosinophils, and plasma cells. In an earlier study, eosinophilic myositis due to *Sarcocystis* infection reported in cattle, sheep, pigs, and horses (Dubey et al., 1989). Valinezha et al. (2008) also reported infiltration of inflammatory cells like lymphocytes, macrophages, plasma cells, eosinophils, fibroblasts, and connective tissues around the degenerated cysts. One of the reasons for increasing in mononuclear cells around the vessels may probably be the host response to the antigen released from the sporozoite or immature schizont (Yarim et al., 2004; Radostits et al., 2007). The presence of antigens released by the parasite reported as a

reason for the infiltration of mononuclear cells (Oryan et al., 1996).

Conclusions

The present work demonstrated that laboratory studies on *Sarcocystis* macrocysts in sheep were informative and may found pathologic lesions in the skeletal muscle of sheep.

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Ethical approval

No applicable.

Conflict of interest statement

There is no conflict of interest.

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