

Fate of macrosarcocyst of *Sarcocystis gigantea* in sheep

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Abstract

This study was conducted to detect the fate of macrosarcocysts of *Sarcocystis gigantea* in the tongue and esophagus of naturally infected sheep, via collection of 25 samples, 10 of which showed calcification. The results showed presence of white different size grains on the wall of the pale esophagus, in addition to presence of nodules containing white chalky materials and on cutting by knife produced grunting sound which indicated calcification. Histopathological results showed presence of granulomatous nodules that contained necrotic centers infiltration by inflammatory cells. Some of which were free from zoites in addition to presence of calcium salt precipitation, which represented dystrophic calcification. Eosinophilic myositis appeared in the tongue was associated with ruptured cyst and released zoites in muscular tissue. Some histological sections revealed ruptured macrosarcocyst with thin wall deposited between muscle bundles. In conclusion, this study showed that the fate of macrosarcocysts included the formation of granulomatous nodules associated with dystrophic calcification and dead zoites in esophagus more than that in the tongue.

Keywords: *Sarcocystis gigantea*; Calcification; Macrosarcocyst; Sarcosporidiosis; Bradyzoites.

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مصير الاكياس العينية لطفيلي المكيسات العضلية في الاغنام

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الخلاصة

تضمنت هذه الدراسة التحري عن مصير الاكياس العينية لطفيلي المكيسات العضلية في لسان ومرئ الضان المخمجة طبيعياً من خلال جمع ٢٥ عينة، ١٠ منها تعاني التكلس. اظهرت النتائج وجود حبات بيضاء مختلفة الاحجام على جدار المرئ الشاحب فضلاً عن وجود عقيدات تحتوي مواد بيضاء طباشيرية عند قطعها بالسكين تصدر اصوات قرقرة تشير الى وجود التكلس. اظهرت النتائج النسجية المرضية وجود عقيدات من الورم الحبيبي ذات مركز متنخر مرتشح بالخلايا الالتهابية والبعض منها خالي من الحويونات فضلاً عن وجود ترسبات من املاح الكالسيوم وهي تمثل التكلس السغلي. كما ولوحظ وجود التهاب العضلة الحمضي في اللسان مع تحطم الاكياس وتحرير العديد من الحويونات في النسيج العضلي. بينت بعض المقاطع النسجية وجود الاكياس العينية محطمة وذات جدار رقيق يتموضع بين الحزم العضلية. تستنتج هذه الدراسة بان مصير الاكياس العينية هو تكون الاورام الحبيبية مع التكلس السغلي وموت الحويونات وفي المرئ كان اكثر وضوحاً منه في اللسان.

Introduction

Sarcosporidiosis is caused by protozoa of the genus *Sarcocystis*, which is a member of the family Sarcocystidae. These parasites commonly and

characteristically cause a chronic, subclinical infection in cardiac and skeletal muscle of livestock by forming macrosarcocysts (Sarcocysts) or macrocyst in the striated muscle of sheep, and infected carcasses may be rejected from export or condemned for human consumption (1,2).

Abattoir surveys have recorded infections by macroscopic cyst of *S. gigantea* in 94.8% and 100% of ovine examined in Mosul (2,3). Infections by macroscopic cyst have been shown to be transmitted by cat (4). The mature sarcocysts are surrounded by primary cyst wall, which derives from the original membrane of the parasitophorous vacuole and it is covered by host cell material. This layer is called secondary cyst wall, or may be also formed by defense cells of the host and may later induce the calcification of the muscle fiber containing the parasite (5).

The aim of this study was to exam the fate of the sarcocysts of *S. gigantea* in eosophagus and tongue in naturally infected ovine.

Materials and methods

Twenty five samples of sheep eosophagus and tongue naturally infected with macrosarcocysts of *S. gigantea* were collected from Mosul abattoir. Ten of them were calcified, their grunting sound was heard after cutting by knife, then fixed in 10% neutral buffered formalin, then after 48-72 hr dehydrated by ethyl alcohol 70-100%, cleaning by xylol and embedded in paraffin wax, to prepare blocks, thus by microtomes cutting section at 4-6 μ m thickness, stained with H&E (6) other's staining with Von gossa's and Al-Zarine stain (7).

Results

Samples of infected eosophagus and tongue showed white rice-grain sized nodules, nodules including calcified cheesy content and after cutting by knife grunting sound was heard (Fig. 1) other samples showed pale eosophagus with round calcified nodules (Fig. 2).



Fig. 1: Sheep eosophagus infected with *S. gigantea* showed white rice-grain nodules (arrow).



Fig. 2: Sheep eosophagus infected with *S. gigantea* showed pale eosophagus, presence of round chronic calcified nodules (arrow).

Histopathological sections of the tongue showed presence of different size of elongated sarcocysts between muscle bundles each one surrounded by thin layer of muscle fibers which contain numerous bradyzoites. Some of these macrosarcocysts ruptured and released merozoites (bradyzoites), area of which infiltrated by inflammatory cells represented by eosinophils (Fig. 3-5), some other sections revealed necrosis and dystrophic calcification in additions to degeneration of muscle fibers (Fig. 6).

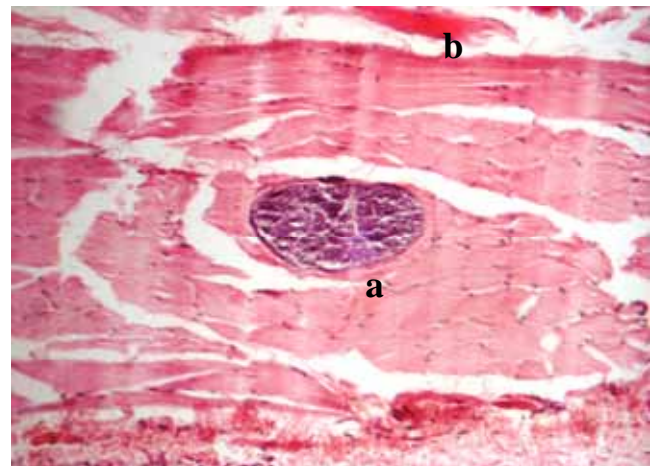


Fig. 3: Histological section of tongue infected with *S. gigantea* showed macrosarcocysts (a) hyaline degeneration of muscle fiber (b) (H and E stain 165X).

In esophageal sarcosporidiosis, histopathologically, revealed typical granuloma, calcified myofibers were locally surrounded by macrophage and numerous inflammatory cells and multinucleated giant cells (Fig. 7,8).

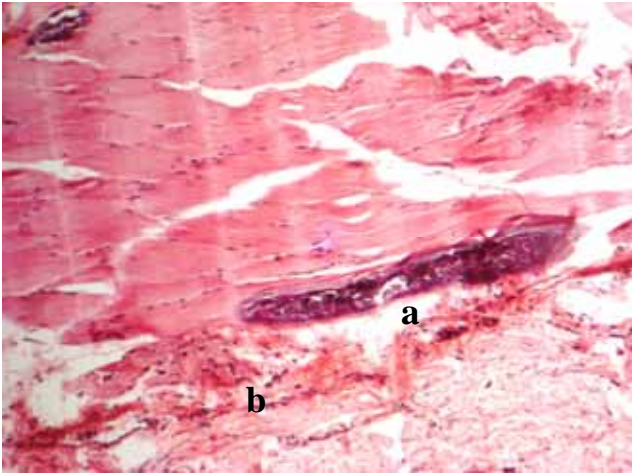


Fig. 4: Histological section of tongue infected with *S. gigantea* showed elongated macrosarcocysts (a) surrounded by eosinophils infiltration (b) (H and E stain 165X).

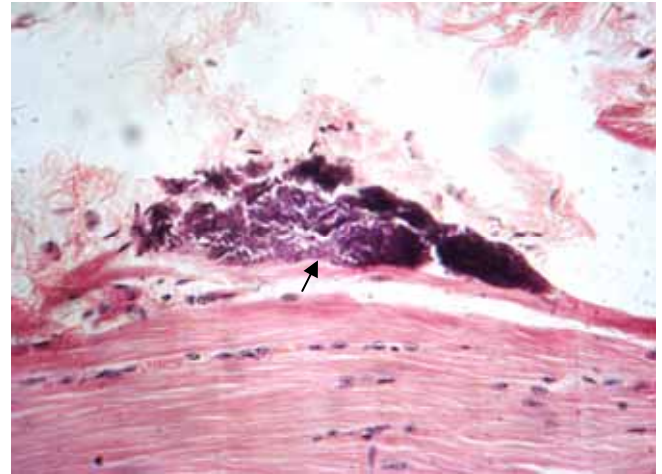


Fig. 6: Histological section of tongue infected with *S. gigantea* showed necrotic and calcified macrosarcocysts (arrow) (Al-zarine stain 370X).

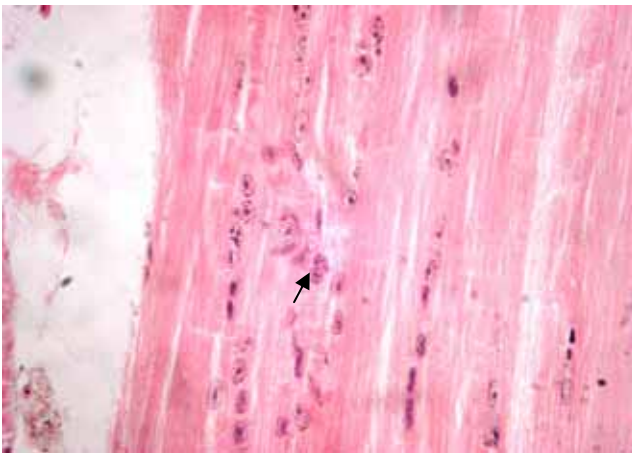


Fig. 5: Histological section of tongue infected with *S. gigantea* showed merozoites (bradyzoites) releasing between muscle bundles (arrow) (H and E stain 560X).

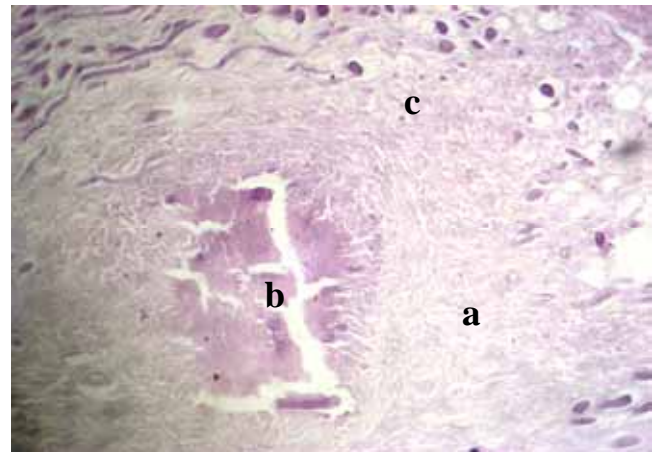


Fig. 7: Histological section of esophagus infected with *S. gigantea*, showed granulomatous nodules (a) contain necrotic centers (b) surrounded by fibrous septa (c) (H and E stain 115X).

In some lesions massive fibrous septa with hyaline degeneration, sarcoplasm fragmentation and mastolysis were observed (Fig. 9), some other sections showed thin cyst wall and necrotic area with dystrophic calcification in muscle bundles. Some macrocysts showed thin cyst wall present between muscle fibers (Fig. 10, 11) which was more evidence in esophagus than in tongue.

Discussion

The presence of a *Sarcocystis spp.* Infection as cysts in muscle termed sarcosporidiosis, is differentiated from the clinical syndrome expressed prior to cyst formation,

referred to a sarcocystosis (8). The *S. gigantea* is very common in Iraq (9), this high prevalence suggests that either sheep are reinfected continuously or that sarcocysts persist for several years in sheep, reinfection is more likely to be the case because the environment is highly contaminated with sporocyst (10) and the severity of natural field infections may therefore be limited by certain host/parasite interaction. The results of this study showed presence of macrosarcocysts of *S. gigantea* in different size in muscle fibers of tongue and esophagus (Fig. 1) (1). These results indicated that infection was in chronic form, in addition to color and size confirmed the chronicity and activity of the cyst. Histopathological changes in ovine

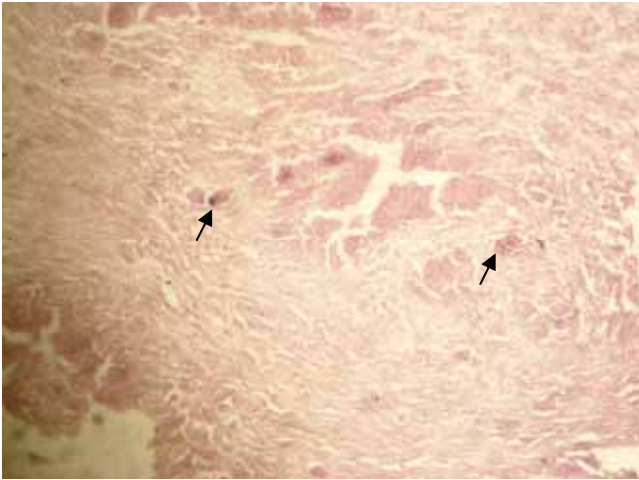


Fig. 8: Histological section of esophagus infected with macrosarcocysts, of *S.gigantea* showed granulomatous nodules surrounded by giant cells (arrow) (H and E stain 115X).

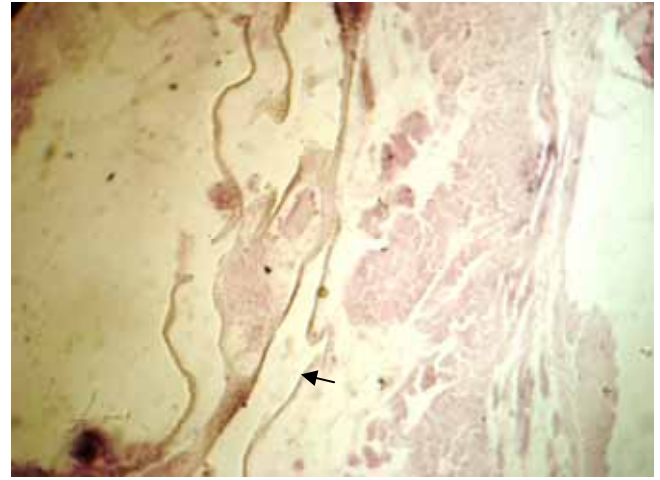


Fig. 10: Histological section of esophagus infected with macrosarcocysts, of *S.gigantea* showed thin cyst wall of macrosarcocysts in muscular layer (arrow) (H and E stain 90X).

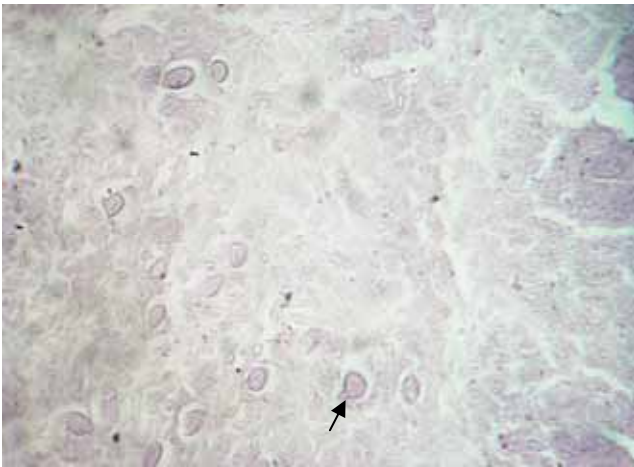


Fig. 9: Histological section of esophagus infected with macrosarcocysts, of *S.gigantea* showed mastolysis (arrow) in necrotic area of macrosarcocysts (H and E stain 370X).

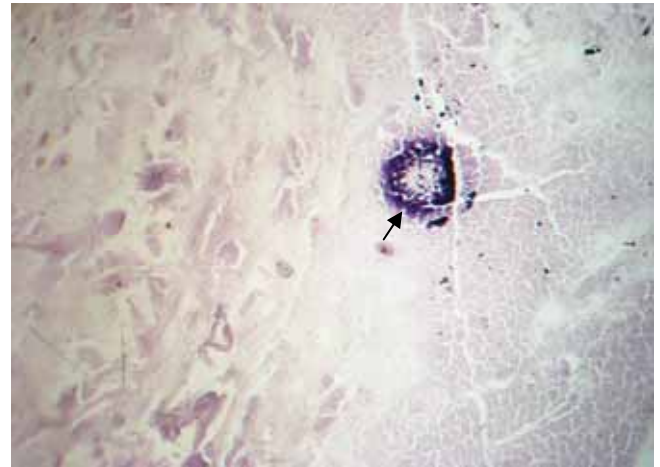


Fig. 11: Histological section of esophagus infected with macrosarcocysts, of *S. gigantea* showed dystrophic calcification (arrow) in granulomatous nodules (Von gossa 90X).

tongue infected with macrosarcocysts of *S. gigantea* showed presence of macrosarcocysts between muscle bundles some of which were ruptured, released bradyzoite concomitant with eosinophilic response in addition to presence dead bradyzoites associated with dystrophic calcification. The results were observed indicated the presence of heavy and chronic infection with *Sarcocystis* spp. (11).

The present investigation provides evidence that chronic infection with *S. gigantea* induce body response in the intermediate host, namely increase the macrosarcocysts in

sized (12). Evidence of ruptured cyst was similar to previous study (13) who reported that some sarcocysts probably rupture from time to time and thus the antigenic stimulus for antibodies production smaintained. Sarcocysts and enclosed organism are removed by leukocytes an area occupied by sarcocysts may be mineralized. The muscle cyst are prone to become ruptured due to enlargement, after rupturing of the cyst, some liberated large zoites, may become immobilized after binding with the apical complex associated antibody in the immunized host and change to round form (Fig. 2) (14). Binding of the surface membrane

and cytoplasmic granules to the corresponding antibody possibly leads to attacks by the eosinophils and lymphocytes on the exposing aspect with some zoites escaping the killing effect, further binding with this antibody intermixed with inflammatory cell debris (15).

Muscle degeneration, myolysis, necrosis and dystrophic calcification other lesions described in our study are consistent with lesions found previously in tongue, oesophagus and skeletal muscle (16) who reported that muscle degeneration and necrosis are common sequelae to myofiber injury regardless of its causes (chemical, metabolic, traumatic and infections) myofiber degeneration can be reversible, if injury progresses beyond the point of no return degeneration become irreversible and necrosis will follow muscle appear pale (Fig. 2). If the calcification is extensive and severe muscle show glistening, white chalky foci, that observed as granting sound, eosinophilic infiltration, hyaline degeneration of muscle fiber, fiber necrosis, dystrophic calcification and sarcosporidiosis, these parameters were increasingly detectable along with growing age of animals (17). Evidence of granulomatous reactions and dystrophic calcification in esophagus were observed in 10 samples, these lesions were correspond with those found previously (18,19), they reported that sarcocysts in granulomas were open and degenerating, whereas those in normal myofiber were infect and non reactive. Other explanations for granulomas lesions include immunologic responses, such as type I hypersensitivity, type II (cytotoxic reactions), and type IV (cell mediated immunity). Each of these mechanisms has some or all of the component necessary for development of these lesions. In the present study granulomas, dystrophic calcification and ruptured cyst were observed in (Fig. 11).

Conclusion of these results indicates that the fate of sarcocysts are formation of granulomatous concomitant with dystrophic calcification and dead of trophozoite in esophagus more than in the tongue.

References

1. Odonoghue, P.J, Ford, GE. The prevalence and intensity in of *Sarcocystis spp.* Infection in sheep. Aust Vet J.1986;63:273-278.
2. Al-Hyali NS. Seroprevalence and toxicity of *Sarcocystis gigantea* different hosts in Nineva Province. [dissertation]. College Vet Med, Univ Mousl, Mosul, Iraq, 2006.
3. Thanoon HB. Oral lesions and their prevalence of local sheep in mosul area. [master's thesis]. University of Mosul, 2009.
4. Rommel M, Heydorn AO. Bertrage zum lebenszyklus der sarkosporidien III. *Isospora hominis* (Railliet und lucet,1891) Wenyon 1923 eme Dauerform der sarkosporidien des Rindes und des Schweins. Berl Munch Tieraerztl Wochenschr.1972;85:143-145.
5. Encyclopedic Reference of Parasitology. Tissue cyst. 2008 1-3. Available from: <http://parasitology.informatik.un-wuerzburg.de>
6. Luna LG. Manual of histologic staining methods of the armed forces, institute of pathology 3rd ed. Mc Graw-Hill Book company, New York, USA. 1968:1-64,236p.
7. Boncroft JD. Histological techniques. 2nd ed. Butterworths of London and Boston. 1975:213-215p.
8. Ford GE. The significance of the Sarcocystosis and Sarcosporidiosis in domestic animals. Aust Adv Vet Sci.1980:61-63.
9. Althah MH. Epidemiological study on Sarcocystosis in human and domestic animals. [dissertation]. College Vet Med, Univ Baghdad, Baghdad, Iraqi, 2002.
10. Dubey JP, Fayer R, Speer CA. Experimental *Sarcocystis hominis* infection in cattle: lesions and ultrastructure of sarcocysts. J Parasitol. 1988;74:875-879.
11. Al-Sultan II, Daoud MS. A case of massive mixed bovine infection with *Cysticercus bovis* and *Sarcocystis spp.* Iraqi J Vet Sci.1998; 11(2):273-274.
12. Munday BL, Obendorf DL. Development and growth of *Sarcocystis gigantea* in experimentally infected sheep. Vet Parasitol.1984;15:203-211.
13. Fayer R, Dubey JP. Bovine Sarcocystosis. Compendium Food Animal.1986;8:130-142.
14. Herzenberg AM, Barta JR, Desser SS. Monoclonal antibodies raised against coccidia and malarial parasites recognize antigenic epitopes found in lankesterellid and adeleorin parasites. J Parasitol. 1995;18: 543-548.
15. Sukhum BMD. The *Sarcocystis* cyst containing beef and pork as the sources of natural intestinal sarcocystosis in Thai people. J Med Assoc Thai.2007;90(10):2128-2135.
16. Mc Gavin DA, Valentine BA. Pathologic basis of Veterinary diseases 4th ed. Elsevier Science publishing. New York. 2008:973-1040p.
17. Bergmann V, Grafe A. The pathomorphology and pathogenesis of acute cardiovascular failure in swine. 1 Histopathologic findings in the myocardium of swine of different age groups. Arch Exp Veterinar Med.1990;44(5):503-512.
18. Jensen DVM, Alexander AF, Dahlgren RR, Jolley WR, Marquardt WC, Flack DE, Bennett BW, Cox MF, Harris CW, Hoffmann GA, Troutman RS, Hoff RL, Jones RL, Collins JK, Hamar DW, Cravans RL. Eosinophilic myositis and muscular Sarcocystosis in the carcasses of slaughtered cattle and lambs. Am J Vet Res.1986;47(3):587-593.
19. Do SH, Jeong DH, Chung JY, Park JK, Yang HJ, Yuan DW, Jeong KS. Eosinophilic myositis in a slaughtered Korean native cattle. J Vet Sci.2008;9(4):425-427.