Fate of macrosarcocyst of *Sarcocystis gigantea* in sheep

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(Received November 1, 2009; Accepted June 30, 2010)

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 macrocyst with thin wall deposited between muscle bundles. In conclusion, this study showed that the fate of macrocysts included the formation of granulomatous nodules associated with dystrophic calcification and dead zoites in esophagous more than that in the tongue.

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

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مصیر الاکیاس العینیة لطفیلی المکیسات العضلیة فی الاغناش

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الخلاصة

تضمنت هذه الدراسة التحییا عن مصير الاکیاـس العینیة لطفیلی المکیسات العضلیة فی لسان ومرئی الضعان المخمّة طبیعیاً من خلال جمع 25 عینة، 10 منها تعیین التکلمس. اظهرت النتائج وجود حبات بيضاء مختلفة الاحجام على جدار المرئі الشامب فضلاً عن وجود عقدات تحتوي مواد بيضاء طباشيریة عند قطعها بالسکین تصدیر أصوات فرقعة تشير الى وجود التکلمس. اظهرت النتائج النسجیة المرضیة وجود عقدات من الورم الحیبی ذات مركز متنخیر بخلایا الالتهابیة وعصب منها خالی من الحوییات فضلاً عن وجود ترسبات من املاح الكالسیوم وهي تمثل التکلمس السلی. كما ولاحظ وجود التهاب العضلة الحمضی فی اللسان مع احتمال الاکیاس وتحرير العديد من الحوییات فی النسيج العضلی. بینات بعض المقاتات النسجیة وجود الاکیاـس العینیة محتمل ولكن نادر رقیق يتم وضع بين الارام العضلیة. تستنتج هذه الدراسة بان مصير الاکیاـس العینیة هو تكون الارام الحیبیة مع التکلسم السغلى وموت الحوییات وفی المرئی كان أكثر وضوحاً منه فی 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 heared 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 µm 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).](image1)

![Fig. 2: Sheep eosophagus infected with *S. gigantea* showed pale eosophagus, presence of round chronic calcified nodules (arrow).](image2)

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 infilterated 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).](image3)

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

In some lesions massive fibrous septa with hyaline degeneration, sarcoplasma 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 *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 eosophagus infected with macrosarcocysts, of *S. gigantea* showed granulomatous nodules surrounded by giant cells (arrow) (H and E stain 115X).

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

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

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

tongue infected with macrosarcocysts of *S. gigantean* showed presence of macrocysts 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 attack 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, easily palpable, rubbery, chalkey foci, that observed as granting sound, easily palpable, rubbery, that associated with dystrophic calcification and sarcosporidiosis, these parameters were increasingly detectable along with growing age of animals (17). Evidence of granulomatous reactions and dystrophic calcification in oesophagus 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


