The effect of macrocysts for *Sarcocystis* spp. in muscular tissue of water buffaloes (*Bubalus buball*s)

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Abstract

Sarcocystosis in water buffalo is caused by eating of contaminated vegetables or water with sporocysts of *Sarcocystis* spp. it's forming two types of sarcocysts are macrocysts or microcysts in their muscles , The aim of current study is to detect effect of macrocysts on the muscular tissue of infected water buffaloes . Histological examination carried out on infected oesophageal muscle of five , more than four years water buffaloes. The histological examination was showed presence of inflammation , hypertrophy and necrosis in the affected muscle tissue , addition to loss of the nuclei in the muscle cells that containing the macrocysts of *sarcocystis* spp. This is the first study for Histological effect of macroscopic *Sarcocystis* spp. infecting muscles of Iraqi water buffaloes .

الخلاصة

يحصل مرض Sarcocystosis في الجواميس بسبب تناول الخضروات أو شرب المياه الملوثة بالأكياس السبورية لطفيليات *Sarcocystis* spp. ، إذ تكّون شكلين من الأكياس هما الأكياس العيانية والأكياس المجهرية في عضلاتها ، هدفت الدراسة الحالية إلى تحديد تأثير الأكياس العيانية في الأنسجة العضلية للجواميس المصابة . اعتمدت الدراسة الحالية على الفحص النسيجي لعضلات المريء المصابة ، لخمسة من الجواميس الأكبر من أربعة سنوات . أكدت هذه الدراسة بوجود التهاب وتضخم ونخر في الأنسجة العضلية المصابة فضلا عن فقدان الأنوية من الألياف العضلية المحتوية على الأكياس العيانية لطفيليات *Sarcocystis* spp. . تعد هذه الدراسة الأولى التي تشخص التأثير المرضي للأكياس العيانية في عضلات الجواميس في العراق .

Keywords : Macrocysts in muscular tissue, Sarcocystosis in Iraqi water buffaloes

Introduction

Sarcocystis species are protozoan obligate parasites , The life cycle of this genus depended two hosts species to complete. The definitive hosts from carnivores such as dogs and cats, and the intermediate host from herbivores such as sheep and cattle

and buffaloes, causing a disease of muscle called Sarcocystosis (Dubey *et al* ., 1989). The vegetables , herbs and water contaminated with sporocysts the primary source to infection intermediate host (Fayer , 2004), the meats infected with cysts is an important source of infecting definitive hosts (Arness *et al* ., 1999). The shape of the cysts may be fusiform or oval in the muscles infected such as the heart , muscles of the neck , face, diaphragm, and could be carried in the muscles of the entire body in extreme cases (El-Seify *et al* ., 2014). Two sizes of cysts in the muscle , macrocyst is large size can be see it by eye, and another size microcyst that sees only by the microscope (Bunyaratvej *et al* ., 1982). Use Anticoccidial treatments that highly effective to preventing disease and protecting calves from injury, there is no final cure for muscular inflammation , but studies continue on laboratory animals (Leek *et al* ., 1980 &1983).

Material and methods

Macrocysts were Identified in muscle tissue by the naked eye after slaughtering an animal, some macrocysts located on the esophagus surface easily Identified as shows in figure 1 , but some others of the macrocysts cannot Identified only by dissection of muscle tissue due to their extension to muscle layers as in shows in figure 2 .

one cm3 of infected muscle tissue was taken from the region were the cysts are located and one cm3 of control muscle tissue was taken from the region non infected , and then fixed in an Boun's solution (750 ml of saturated aqueous with Picric acid ; 250 ml formalin and 50 ml of Glacial acetic acid) for 24 hour (Humason , 1972) .

Fixed Samples were transferred directly into the alcohol 50% and replaced several times and then transferred to the alcohol 70% then 80% , then samples left for two hours and then alcohol 90% , left two hours repeated twice and then alcohol 100% to ensure dewatering of samples . Clearing process was completed by using xylene alcohol 1:3% , 1:1 and 3:1 .Then left two hours for every pass (Luna , 1960).

Used the wax bath to Infiltration and Embedding , used paraffin wax melting degree 56-60 for 90-120 minutes in each basin , then poured the samples in plastic molding molds. Microtome was used to cut the blocks at 5-7 Mm thickness once cut , the tissue sections were carefully transferred to a warm water bath 40 Cº, then put slices on a glass slide with Meyer's (Abu- Aqleh ,1999) , then allowed to dry on warming plate for 24-48 hours, 50 Cº.

Dye the slides by using Heamatoxylin - Eosine (Luna, 1960) , the slides put in xylene for two minutes and then pass on 100% alcohol then 90% and 70% for 2 minutes for each concentration and then placed in distilled water for two minutes , then immerse in dye Heamatoxylin for five minutes and then washed with Tap water, then put in an acid alcohol (5-7 drops of HCL + 100 ml alcohol 70%) for a

second, then washed with distilled water and then in the Eosine dye for less than a minute and then washed with distilled water and passed on Xylene 1 and Xylene 2 70%, 90% and 100% respectively. Downloaded forms using Canada balsam and then put the cover slide on each form and left to dry for 24 -48 hour and examined by using optical microscopy.

Figure 1. Part of water buffalo esophagus showed macrocysts located on the surface of the esophagus muscle**.**

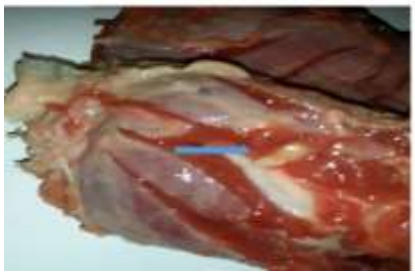

Figure 2. Part of water buffalo esophagus showed macrocysts extended through muscle layers.

Results

The results of the current study have recorded pathological signs in infected muscle tissue, three satisfactory marks in muscle in the regions presence of the parasite macrocysts when compared with the control samples , (figure 3). Figure 4 shows inflammation in infected muscle. Figure 5 shows presence of necrosis with large numbers of bradyzoit (b) . Figure 6 shows Hypertrophy of cells and loss of nuclei.

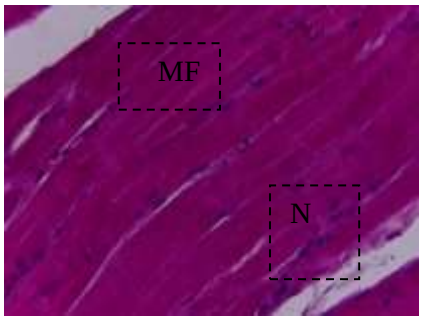

Figure 3 . Longitudinal section of control sample 40X. Nucleus(N) , Muscular fibres (MF)

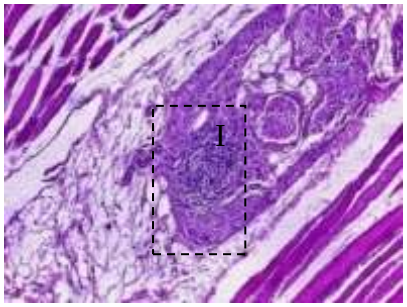

Figure 4 . Longitudinal section in infected muscle showed inflammation (I) in muscle tissue 10X.

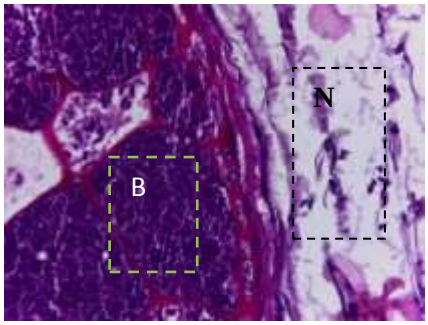

Figure 5 . Cross section in infected muscle showed presence of necrosis (N) with large numbers of bradyzoit (B) 40X.

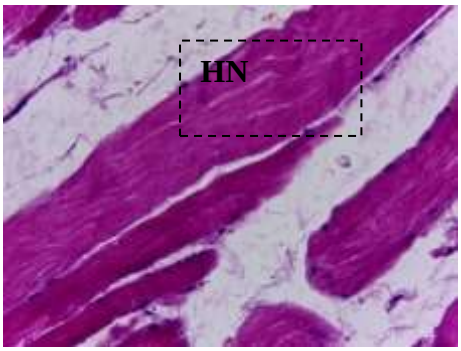

Figure6: Longitudinal section in infected muscle showed Hypertrophy of cells and loss of nuclei (HN) 40X.

Discussion

Water buffalo meat can be infected with two species of *Sarcocystis* produce macrocysts : *S. buffalonis* and *S. fusiformis* , and two species produce microcysts : *S. levinei* and *S. dubey* (Dubey *et al*., 1989).The histological study record three pathological signs in infected meat with macrocysts of *Sarcocystis* spp. are inflammation , presence of necrosis with large numbers of bradyzoits and having Hypertrophy of cells and loss of nuclei , for this reason the infected meat is quality poor and unsuitable for eating and the muscle tissue have sarcocysts when consumed from a carnivore (the definitive host) , will cause intestinal sarcocystosis. The microcysts have pathogenic and cause acute diseases presented by fever,

abortion , anorexia and anaemia in early period of infection and then may be develop some chronic disorders . On the other side , macrocysts can be affect the meat marketing and quality and cause economic loss (Pescador *et al* ., 2007 ; Tenter , 1995).

Conclusion

The current study has shown that macrocysts have histopathological in muscular tissue of water buffaloes changes have been detected , it can be cause economic loss.

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