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Histopathological diagnosis for *Sarcocystis spp.* in slaughtered sheep and goats in Misan governorate/Iraq

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***Abstract*---**This study aimed to determine and identify *Sarcocystis spp.* infection in sheep and goats in Misan governorate, Iraq. samples from oesophagus, diaphragm, tongue, and heart muscles were examined histologically for microscopic and macroscopic cyst , 70 out of90 sheep 77.78 % had microscopic thick walled sarcocysts with mean 53 ×24.72 µm diagnosed as *Sarcocystis tenella* while 25 out of them 27.78 % had microscopic thin-walled sarcocysts with mean 49.45×33 µm diagnosed *Sarcocystis arieticani*s , esophagus showed the highest infection rate above all organs examined for thick – walled Sarcocystis while diaphragm was the highest for thin –walled Sarcocystis , the heart was the lowest infected organ for both type as 27.78% and 3.33% respectively . In goats 50 sample examined revealed 20 infected with thick walled sarcocysts only with different in distribution according to organs being high in esophagus 40% and lowest in heart 16% with mean measurement 60.61 × 37.75 µm diagnosed as *Sarcocystis capracanis*. In sheep histopathological changes show various sizes and shapes of microcysts, including those that are round, oval, or elongated with inflammatory reaction around the cysts of infected muscle fibers in sheep but no histopathological changes appear in goats using Periodic Acid-Schiff (PAS) stain and Haematoxylin and Eosin (H& E). Histological examination revealed the presence of *S. gigantea* macrocyst with (H & E) in 2% of sheep and 2% in goats s. histopathological changes show inflammatory reaction around the cysts of infected muscle fibers in sheep and goats s.

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***Keywords*---**sheep, goats s, Misan, histopathology, Sarcocystis spp.

Introduction

Sarcocystis species are intracellular protozoan of the phylum Apicomplexa Currently, 200 *Sarcocystis species* have detected with a strict host and genus specificity. life cycle *Sarcocystis* spp. develop in a two host, intermediate and a definitive host, humans can serve as an intermediate host and definitive host for different *Sarcocystis* species. consequently, humans are definitive hosts when they consume tissue cysts with undercooked meats (sarcocysts) (Al-Hayali and Daood, 2002).

Sheep serve as the intermediate host for the mostly pathogenic *S. arieticanis* and *S. tenella* organisms that are spread by dogs. These parasites cause microsarcocysts to form in the skeletal, cardiac, and central nervous systems. (Saki *et al* 2010). Domestic goats (Capra hircus) serve as intermediate hosts for the cyst-wall-structure-based morphological differentiation of the *S. capracanis*, *S. hircicanis*, and *S. moulei* pathogens. Canids transfer microscopic sarcocysts produced by *S. capracanis* and *S. hircicanis*, while felids transmit large cysts from *S. moulei* (Heydorn and Matuschka, 1981).The species of Sarcocystis are generally low pathogenicity in the definitive host, while can cause severe clinical signs in intermediate hosts, like fever, weakness, inappetence, anemia, weight loss, hair loss, hemorrhagic and encephalitis, nervoussigns have also been described in sheep and goats including alteration of behavior, stupor, ataxia, opisthotonos, nystagmus and galloping gait (3first). Other signs have been reported, including exophthalmia, sialorrhea, submandibular oedema, haematuria, and urination disorders in acute sarcocystis infections of prenatal females cause fetal death, abortion. The size, form, and wall thickness of the cyst, along with other physical characteristics, are typically used to identify the species of Sarcocystis. (Lau *et al* 2014).

Due to a lack of information on the incidence of *Sarcocystis spp.* in sheep and goats in Misan governorate our present study was performed to detect Sarcocystis species were found in sheep and goats that were slaughtered in an abattoir in the Misan governorate, which is in the southern part of Iraq and describe morphometric of microscopic and macroscopic cysts of *sarcocystis spp.* in sheep and goats in different organs.

Materials & Methods

Collection of samples

Ninety slaughtered sheep and 50 slaughtered goats were randomly examined from abattoir and those animals slaughtered from butchers' markets, in Misan, different body tissues (tongue, esophagus, diaphragm, heart) of each animal were collected during the study period from December 2020 to August 2021. Collected samples put in clean labeled bags.

Examination of the collected samples

Fresh tissues Samples from different tissue and grossly examined tissues by the naked eye for macroscopic Sarcocysts fine needle and thin forceps to separate the cysts from any attached muscle fibers. The specimens were then fixed by soaking them in 10 percent neutral buffered formalin for (24–48) hours at room temperature, cut into 0.5 cm-thick sections, and dehydrated with serial ethanol dilutions and cleared with xylene. They were then embedded in paraffin, sectioned into (4–5) micro The light microscope with oil immersion was used to analyze the wall morphology of the macrocysts and microcysts in the stained sections. (×100), dimensions of each sarcocyst and wall thickness was measured using ocular micrometer, *Sarcocystis species* identified on the basis as described by Levine (1986). Images were captured using digital camera.

Statistical Analysis

The Statistical Analysis System was used to detect the effect of difference factors in percentage of study using Two-Way ANOVA test followed by Tukey's multiple comparisons test ($p< 0.05$). (SAS, 2012).

Results and Discussion

Histological analyses showed the existence of both microscopic and macroscopic cysts by light microscope 70 out of 90 sheep 77.78 % hadthick walled sarcocysts (*Sarcocystis. tenella)* while 25 out of them 27.78 % had thin-walled sarcocysts (*Sarcocystis arieticanis*). (Table 1).These results are in accordance with study of (Abdullah, 2021) in Iraq who demonstrate with his histopathological examination percentage 92.5% in slaughtered sheep with morphologically Thin-walled and thick-walled microsarcocysts, two different varieties, and (Beyazit *et al* 2007) who differentiated from surveyed study using digestion and histological examination of *Sarcocystis* of 200 sheep in Izmir province ,two types of microcysts were Sarcocystis *ovicanis*, and *Sarcocystis arieticanis*, and considered it responsible species for ovine sarcosporidiosis in Izmir. Most researchers observed that the predominant species in sheep was thick walled sarcocyst (*S. tenella*). Hussein *et al* (2018) examined 104 sheep from different organ samples (esophagus and heart muscles) identifying it as *Sarcocystis tenella* also study of Arshad *et al* (2007) recorded that the prevalence of *Sarcocystis tenella* was found in 100 % of the all organs. Nedjari (2002) demonstrated that in Algeria, *S. tenella* predominated 60.63% to S. arieticanis 39.36 %.

The distribution of *Sarcocystis. tenella* was 75.56% for diaphragm, 66.67% for tongue, and 27.78% in heart, the most infected tissue with this species was esophagus (77.7%). *Sarcocystis arieticanis* observed mostly in the diaphragm 27.77 %, followed by esophagus 22.22%, then tongue 11.11%. the lowest prevalence for both species was in heart 27.78% and 3.33% respectively, with no significant difference between the infection of both species in all types of tissues while Comparisons between Thin- and Thick-wall groups of all tissue types is significant at ($P < 0.05$). Table 2.the most infected organ/tissue was esophagus with thick-walled sarcocystis as rate 77.78 % and diaphragm for thin walled sarcocystis disagree with some studies like (Shahraki *et al* 2018), but agree with

study of Mahran (2009) in Egypt, and Asma *et al* (2017) north of Algeria. Additionally, according to Gareh *et al* (2020). the diaphragm follows the esophagus as the muscle in camels with the greatest amount of microscopic sarcocystis infection. The highest rate of infection in esophagus may be due to activity of thetissue and more blood supply then there is more chance to exposure to infection. (El-Mishmishy,2017). Less infection rate in our results was recorded in heart for both type as with rate 27.78%, 3.33% for thick-walled and thin–walled sarcocystis respectively. Similar studies were reported by some authors like Al Quraishy *et al* (2014) that reported lowest infected in heart from slaughtered sheep at Riyadh city. The differences in distribution of Sarcocystis *spp.* in examined organ. may be because of oocysts contamination, differences in nutritional status of the hosts that may lead to variations in the immunity of the host to infection of parasites (Shazly, 2000).

Hematoxylin and eosin stain (H&E) and Periodic acid–Schiff (PAS) stain used to study cysts morphologically; cysts in sheep appeared as spherical, oval, or elongate in shape with a mean size of 53 ×24.72 µm. Different sizes have been discriminated according to organ type. Tables 1and 2. Cyst wall was radially striated with numerous, very crowded palisade-like villar. Figure 1(a, b). These morphological features were found similar to that reported by O'donoghue and Ford, (1986) who described *Sarcocystis tenella* cysts thick wall, striated with villar protrusions appeared somewhat short or stubby. A mean size of thick wall sarcocysts was 53 ×24.72 µm approach to those found by some authors. Tinak, (2009) discovered that *S. tenella* cysts were 68.591 mm long and 23.260 mm wide, but Erber (1982) found that themeasurement of *S. tenella* were 300—650 × 20—50 µm.

Thin-wall sarcocysts that represented as *Sarcocystis arieticanis* were measured 49.45×33 µm with a wall of <1 µm in thickness, Figure 2 (a, b), agree with (Saito *et a*l 1997) which observed by histopathological study that the cyst wall is thin and structureless. Cyst dimensions were 49.45 33 µm, matching those previously documented for this parasite. El-Morsey *et al* (2019) and Haziroglu *et al* (2002) showed that the cysts were ranging at (52.5—162.5) µm in length and (35.0—62.5) µm in width. Thick measurement of *Sarcocystis. tenella* was classified into three groups ranged between (1-4) µm, on the other hand; the most observed sarcocysts rangedfrom (1.5-2) in thickness from 200 cyst examined and the less one observed was in range (2.5-4) which was (15) cyst only with significant differences between them. Different distribution thick wall of sarcocysts in tissues was observed with no significant differences between groups Table 4. These results were similar to some researchers' observations that based on the size of the cysts determined the Sarcocystis species. (length, diameter, thickness). (Vercruysse and Van Marck (1981), and Odening *et al* (1995) recorded thickness from 1.08-3.85 whilst (Dong) indicated that the wall thickness was 0.56 0.54 was 0.56 ± 0.54 µm. Sun (2021) reported that cyst wall measuring was 1-2 µm in thickness.

In goats s, only thick–walled sarcocysts (*Sarcocystis capracanis*) was detected with infection rate 40% out of 50 animals examined, infection rates observed in different examined tissues (esophagus, diaphragm, tongue, heart) were 40%, 30%, 26%, and 16% respectively with no significant differences of infection at (P

< 0.05). presence of only thick–walled sarcocyst in our study was agree withstudy of Zangana and Hussein (2017) which recognize on basis on morphology of microscopic cysts of sarcocysts *S. tenella*, also known as *S. ovicanis*, and *S. capraccanis*, also known as *S. tenella*, were discovered in sheep and goats s, respectively.

Morphological examination of sarcocysts stained with Haematoxylin and Eosin (H & E) and Periodic Acid–Schiff (PAS) stain revealed presence of cysts with mean measurement 60.61 × 37.75 µm, wall of cysts had radial striation with finger-like villar protrusions varied in thickness from (1-3.5), our suspected detection is *Sarcocystis capracanis*, size and wall structure described in our studyapproach to study of Barham *et al* (2005) who point out that *S. capracanis* was present in goats microcysts because they exhibited a striated wall, variable thickness, and tiny finger-like villar protrusions, while Abdel-Rahman (2010) distinct two morphologically Sarcocystis cysts in histological sections, thin and thick-walled cysts.

Most infected tissue was in esophagus as in sheep, these results agreed with results obtained by Barham *et al*, (2005) , Abdel-Rahman (2010) and Kudi *et al* (1991). (Abdel-Rahman (2010) recorded high prevalence of thick-walled cysts in esophagus 87% from overall indicated cysts and disagree of (12) in Iran who found that in cattle, the esophagus (36.6 %) had the lowest contamination and the heart (68%) the greatest, and they attributed this to the fact that different contaminating species prefer different textures for the creation and development of cysts. Histopathological examination of the organs exhibited presence of thick walled sarcocysts between muscle fibers of diaphragm. Figures 4. Infiltrating of inflammatory cells in the area around a sarcocyst. Figure 5. Sections of the goats 's tongue showed different types of thin walled sarcocysts. Figure 6. These findings agreed with studies by Kudi *et al* (1991), Sun *et al* (2021), Rad *et al* (2020), and Mostafa *et al* (2021) who mentioned in his study in Mosul city/ Iraq by use Hematoxylin and Eosin (H&E) and Masson's trichrome stain in diaphragm presence of characteristic histopathological changes in sheep muscles included intense infiltration of inflammatory cells.

At the gross inspection for macrocysts infection 2 from 90 sheep examined (2.22%) and 1 from 50 goats examined (2%) were positive for macrocysts which was detected in the esophagus only. Table 1. Our finding of histological sections of the macroscopic sarcocystis stained with hematoxylin and eosin stain and isolated from the esophagus of sheep and goats revealed that the macrocyst is surrounded by two layers the outer layer is a secondary wall (SW) which is 2.5-4 µm thick and consists of connective tissue characterized by the presence of Spindle or oval nuclei under it located the primary wall (PW). It is inner layer with a thickness of (1.2-2.5) µm, Figure 7; that extends into the lumen of the cyst and separates it into compartments by septa, and the compartments inside them contain bradyzoites crowd peripherally leaving the central area almost empty, Figure 8; and classified according to the shape and measurements to mature and immature bradyzoites. The mature was crescent-shaped with sizes (5-10) µm in length and (1-2) µm, while the immature was circular with size ranging (2.5-5.5) µm in length and its width ranged between (1-2.5) µm in shape.

Based on the morphology cysts, the macroscopic cysts in sheep and goats were

identified as *S. gigantea*. These findings came in close to the results of other studies (Rad *et al* 2021, Abuelwafa *et al* 2016). Many studies have shown that *S. gigantea* was the predominant macroscopic species in sheep and can present in goats (Mahran, 2009). Swar and Shnawa (2020) suggested that sheep and goats can be Cross-infection may also happen between potential intermediate hosts for *S. gigantea* and *S. moulei*, respectively. Histopathological changes of the macroscopic cysts in muscles showed a degenerative reaction, odema and infiltration of inflammatory cells. The infected muscle's vascular lesion includes vascular growth and wall hyperplasia. Figure 9. Study by Valinezha *et al* (2008) noted that the presence of inflammatory cells such as lymphocytes, macrophages, plasma cells, eosinophils, fibroblasts, and connective tissues around the degenerating cyst concur with the findings. *S. gigantea* in sheep was found to have ruptured macrocysts that produced merozoites and necrotic centers that were invaded by inflammatory cells, particularly eosinophils. (AI-Hyali *et al* 2011). In contrast the study by Faghiri (2019) observed no inflammatory reaction surrounding the cysts or surround the diseased muscle fibers. The inflammatory reactions and necrosis of muscles can influence the quality of the meat and that can be related to the toxins that released by the cysts (Singh, 2004).

Table 1: Sarcocystis spp. prevalence in various sheep tissues (N = 90).

Type of tissues	Thick-Walled Sarcocyst Number (%)	Thin-walled Sarcocyst Number (%)
Esophagus	70 (77.78)	20 (22.22)
Diaphragm	68 (75.56)	25 (27.78)
Heart	25 (27.78)	3 (3.33)
Tongue	60 (66.67)	10 (11.11)
Significant (p 0.0084) (Thin- and Thick-wall groups)		

Table 2: Morphological differences between sheep thick-walled and thin-walled sarcocysts.

Type of tissues	Thick-Walled Sarcocyst Number (%)	Thin-walled Sarcocyst Number (%)
Esophagus	70 (77.78)	20 (22.22)
Diaphragm	68 (75.56)	25 (27.78)
Heart	25 (27.78)	3 (3.33)
Tongue	60 (66.67)	10 (11.11)
Significant (p 0.0084) (Thin- and Thick-wall groups)		

Table 3: Thickness of sarcocysts wall in sheep. (N =200)

Thickness of the wall(µm)	Number	
1 - 1.5	30	P= 0.7589 (not significant)
1.5 – 2	155	
2.5 – 4	15	
Total	200	

Table 4: Thickness of sarcocysts wall according to tissues in sheep. (N=200)

Tissues	Thickness (µm)		
	1	1.5- 2	2.5- 4
Esophagus	12	50	6
Diaphragm	10	70	8
Heart	3	10	0
Tongue	5	25	1

Table 5: *Sarcocystis spp.* prevalence in various goats tissues. (N=50)

organ	Thick-Walled Sarcocyst
Esophagus	20(40%)
Diaphragm	15(30%)
Heart	8 (16%)
Tongue	13(26%)

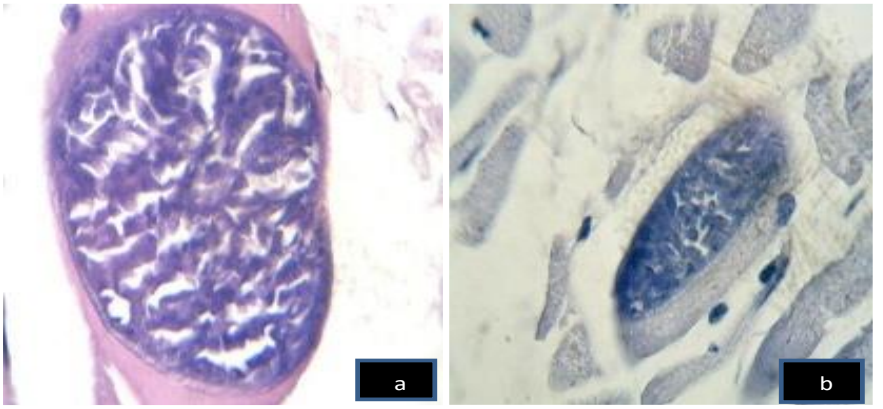

Figure 1: Thick-walled sarcocyst (*S. tenella)* in sheep.
a- H & E stan. b- PAS stain. 100x.

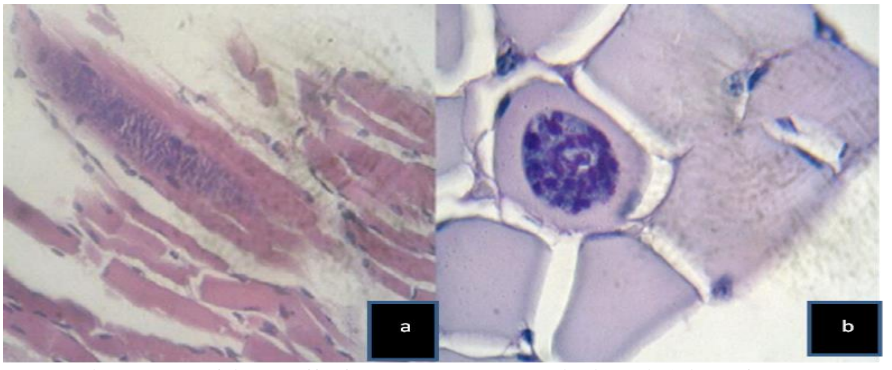

Figure 2: Thin-walled sarcocyst *(S. arieticanis*) in sheep.
a- H&E stain. b- PAS stain. 100x.

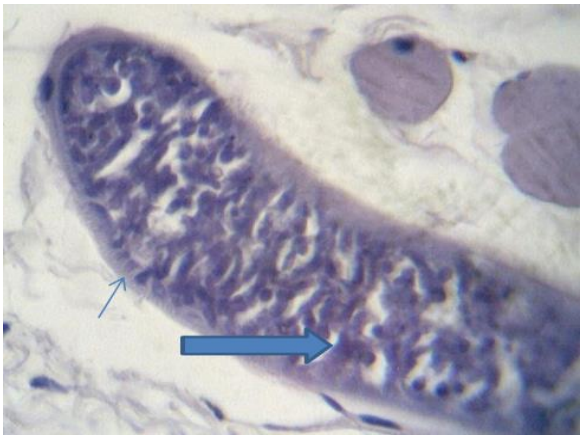

Figure 3: Thick sarcocyst wall (*S. tenella*) with radial striation appearance in sheep (thin arrow), existence of banana-shaped bradyzoites. (PAS stain) (thick arrow). 100x.

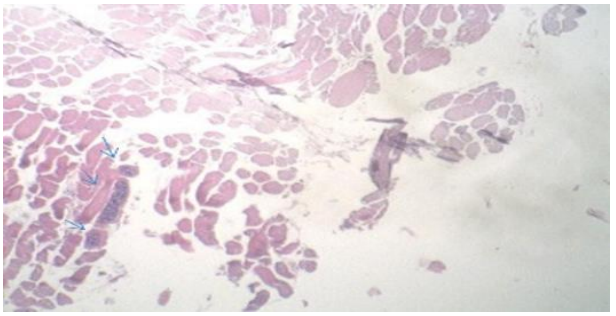

Figure 4: Multiple large round, oval and elongated shape of thick walled sarcocyst (*S. tenella*) in diaphragm of sheep. 4x H&E.

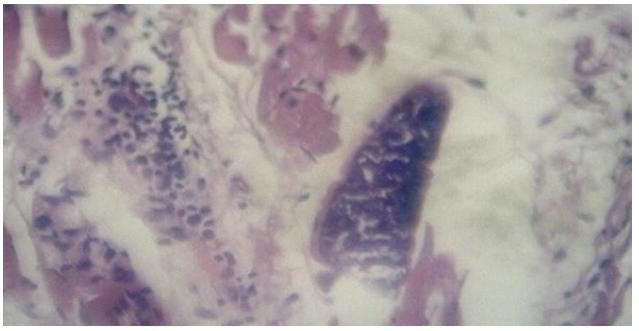

Figure 5: Sheep tongue muscle sarcocyst with an elongated, thin-walled (<1µm) structure with inflammatory cell infiltration. 100x.

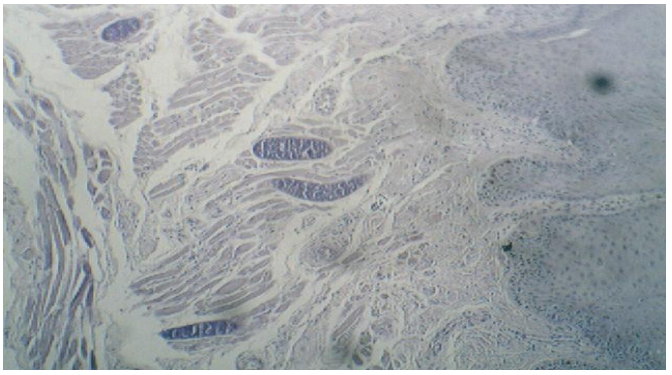

Figure 6: Multiple oval shape thick -walled sarcocysts (*S.capracanis*) in tongue of goats . (PAS stain). 10x.

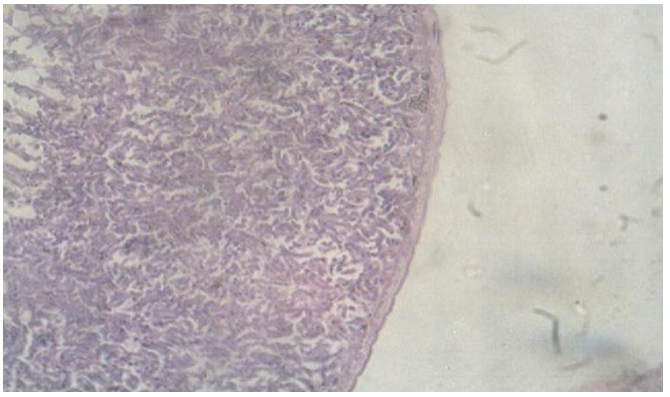

Figure 7: The two-layer macroscopic cyst in sheep with protrusions in the primary wall. (H&E stain).×10.

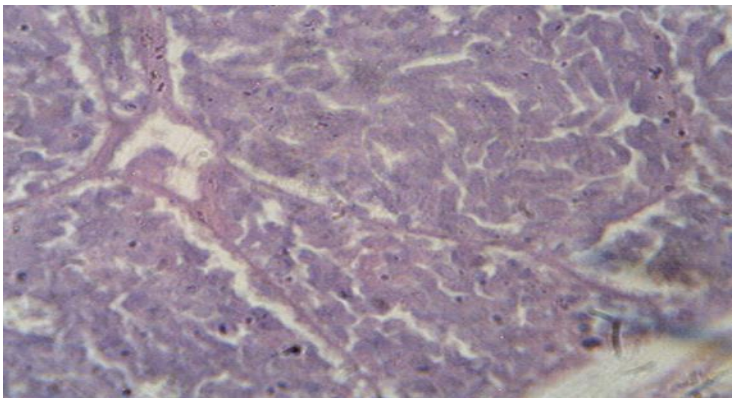

Figure 8: The macroscopic sarcocystis in sheep shows the distribution of mature and immature bradyzoites within the cyst separated by septa. (H&E stain). ×100.

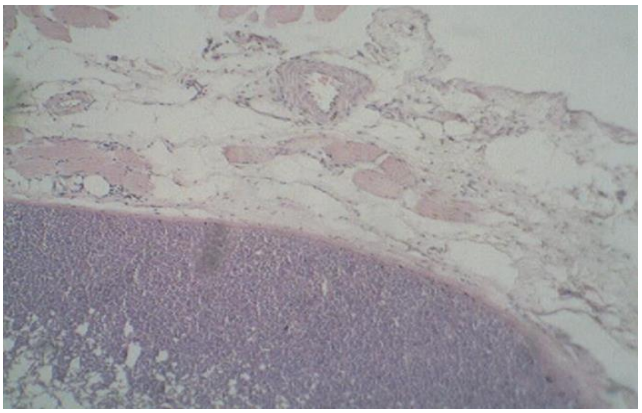

Figure 9: included vascular proliferation and wall hyperplasia in the infected muscle between the affected muscles with infiltration of inflammatory cells. (H&E stain). ×40.

Conclusion

The present study is the first research that recorded infection of *Sarcocystis spp.* in sheep and goats in Misan Governorate/ Iraq. The parasite species that detected in microscopic cysts in sheep and goats were *Sarcocystis arieticanis* and *S. capracanis*. In contrast *S. gigantea* have been reported in macroscopic cysts in sheep and goats.

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