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Macroscopic and Microscopic Examination of Sarcocystosis in Small Ruminants of Duhok Province, Kurdistan Region, Iraq

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ABSTRACT

A total of 2358 sheep and 532 goats were examined for the presence of macrocystis of *Sarcocystis*. For microcysts, different muscle tissues were randomly taken from 118 sheep and 110 goats. Macrocystis was examined through naked eye inspection, while microcysts were examined microscopically by using histopathology, pepsin digestion, mincing & squeezing and muscle squash methods. The overall prevalence of macrocystis was 1.2% in sheep and 2.6% in goats. The intensity rate of cysts was 4 cysts/ gram in sheep & 3 cysts/ gram in goats, respectively. While the overall prevalence rate of microcysts in sheep and goats was 96.5%. The infection rate in sheep was 96.6% and in goats was 96.4%. The total intensity rate of microcysts was 32.4 cysts/ field in sheep and 16.8 cysts/ field in goats, respectively. Histopathological examination found different shapes, size, wall thickness and intensity rate of microcysts in muscle tissues of sheep & goats. The pathological reaction showed mild to moderate granulocytosis and mononuclear cells infiltrated surrounding the microcysts with necrotizing and degeneration of myofibrils. The largest average size of spindle and round shaped cysts (290±89.7 x 76.1±10µm and 88.8±10.3µm) in goats and (127.2±18.9 x 53.3±5.4µm and 74.4±7.5µm) in sheep, was detected in the esophageal muscle. Statistically, there was significant difference ($P<0.05$) in the prevalence of macrocystis in sheep and goats, while no significant difference ($P>0.05$) was observed in the prevalence of microcysts between both animal species.

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INTRODUCTION

Sarcocystis is a zoonotic parasitic disease caused by coccidian protozoa of the family *Sarcocystidae* (Fayer et al. 2015). The parasitic infestation is characterized by cyst forming in different muscle tissues (muscular sarcocyctosis) of the intermediate hosts including skeletal muscle, smooth muscle and cardiac muscles of esophagus, diaphragm, heart and tongue, or colonization of the lamina propria in the small intestine (intestinal sarcocystosis) of definitive hosts including canines and felines. The asexual multiplication stages (merogony & cyst formation) develop only in the intermediate hosts which in nature are often called prey animals while, the sexual stages (gametogony & sporogony) develop in the definitive hosts which in nature called predator animals (Hussein et al. 2023). There are more than 200 species of the genus *Sarcocystis* transmitted among different animal species worldwide. Generally, Sheep can be infected with four *Sarcocystis* species including, *Sarcocystis ovicanis* (*S. tenella*) & *S. arieticanis*, which are pathogenic, microscopic cyst forming and transmitted through canines, while *S. ovifelis* (*S. gigantean*) & *S. medusiformis* are nonpathogenic, macroscopic cyst forming and transmitted through felines (Hosseini et al. 2012). Goats can harbor three different species of *Sarcocystis* such as *S. capricanis* and *S. hircicanis* which produce microscopic cysts, pathogenic and transmitted by canine while *S. caprafelis* (*S. moulei*) produces macroscopic cysts, non-pathogenic and transmitted by feline (Hussein et al. 2025a). In General, the *Sarcocystis* species with canids as definitive hosts are more pathogenic than those species with felids as definitive hosts. In the intermediate host, after ingestion large number of sporulated oocysts or

sporocyst they cause reduced weight gain, weight loss, decrease milk production, abortion as well as neurological signs and even death (Fukuyo et al. 2002). Humans can either act as an intermediate host (Intestinal form) when they ingest food and water contaminated with sporulated oocysts or sporocysts or as definitive host (Muscular form) after consuming raw or undercooked meat with the parasitic cysts containing bradyzoites (Fayer et al. 2015). Diagnosis of sarcocystosis is commonly performed through microscopical examination of muscle tissues containing microcysts from infected intermediate hosts such as skeletal muscle, esophagus, diaphragm, tongue and heart, while macroscopic cysts are visible by the naked eye and can be detected macroscopically. There are several confirmed techniques used for detection of microscopic type of *Sarcocystis* including serological tests, peptic digestion method, mincing and squeezing preparation technique, histopathological examination and molecular characterization (Wu et al. 2022). The main objective of the present study was to determine the prevalence of sarcocystosis in slaughtered sheep and goats in Duhok abattoir/ Iraq, to estimate the intensity rate, the measurement size and morphology of microcysts and macrocystis of *Sarcocystis* in different muscle tissues, and to evaluate the efficiency of different techniques for diagnosis.

MATERIALS AND METHODS

Sample collection

A total of 2358 slaughtered sheep and 532 slaughtered goats were examined in Duhok abattoir/ Iraq by the naked eye for the presence of macroscopic cyst of sarcocystosis in esophagus, diaphragm and heart during period of six months. A total of 472 muscle samples from 118 sheep (Male 72 & Female 46) and 440 muscle samples from 110 goats (Male 99 & Female 11) of ages 1 to 2 years old were collected randomly from different muscle tissues of esophagus, diaphragm and heart. Approximately 100-150g of muscle tissues were put in clean plastic bags and kept in an ice box then transported to laboratory of diagnosis, college of veterinary medicine, and the University of Duhok for further investigations.

Macroscopic examination

All collected muscle samples from different muscle tissues were examined at the laboratory for the occurrence of macrocystis. Cutting sections of muscle tissues were performed by clean scalpel and scissors to visualize the cysts through naked eye and magnification lens under dissecting microscope. Each positive muscle sample with macrocystis was weighed through a standard electronic balance and the numbers of cysts were calculated by cysts/g or cysts/organ for determining the intensity of parasitic infection (Hussein et al. 2023a).

Microscopic Examination

For examination of microcysts in muscle tissues, different techniques were used, including.

Peptic digestion technique

A modified method which was described by Latif et al. (1999) was performed in this technique. Approximately 25g of muscle tissues were minced and put into 50 ml of digested solution (1.3g pepsin, 2.5g NaCl, and 3.5mL HCl in 500mL of distilled water) in a clean conical glass flask. The suspension was mixed well then incubated at 40°C for one hour. The digested suspension was left at room temperature for 15 minutes then filtered through a fine mesh strainer with a double layer of surgical goaz. The sifted solution was put into a clean test tube and centrifuged for 5 minutes at 3000rpm. The supernatant was poured or discarded, and the sediment was re-suspended with 0.5mL of buffered saline (pH 7.2), or distal water. Finally, a drop of the sediment was put on a clean glass slide for detection of free bradyzoites under light microscope (40X).

Mincing and squeezing method

Approximately 5g of muscle tissue sample was finely cut and minced through a sterile scalpel and scissor in a clean glass Petri-dish. 10mL of phosphate buffered saline (PBS, pH 7.4) was added into the minced muscle tissue and mixed well. The homogenized muscle sample was filtered through a double layer of surgical gauze in a fine meshed strainer and a drop was put on a clean glass slide for detection of microcysts under the light microscope (40X) (Hussein et al. 2017). In addition, dry smeared slides were prepared and fixed in absolute methanol for 3 to 5 minutes, then stained by Giemsa 5% for 30 minutes for estimation of the shape, size and intensity rate of free microcysts under the light microscope (100X) (Zangana & Hussein 2017).

Muscle squash method

A modified method of Hussein et al. (2022) was used in this study. Approximately 1 gram of fresh muscle tissue was finely cut into very small pieces (approximately 3-4mm thick). The tissue pressed and crushed firmly between 2 glass slides, then examined under the compound microscope at 40X magnification for detection of microcysts between muscle fibers.

Histopathological examination

According to the protocol performed by Meena et al. (2022), heavy infected muscle tissues of esophagus, diaphragm and heart were fixed in 10% buffered formalin then dehydrated in different concentration of alcohol before embedding into the paraffin wax. The tissue blocks were sectioned at 3µm thick and stained with different stains such hematoxylin and eosin (H and E), periodic Acid Schiff (PAS), and Giemsa stain for morphological identification and estimation of the intensity rate, measurement size, shapes and wall thickness of microcysts under light microscope at 10X, 40X, and 100X magnification.

Intensity rate & Measurement size

Number of cysts/ microscopic field was used to determine the intensity rate of microcysts, and number of cysts/ grams of tissue or number of cysts/ organs was used to determine the intensity rate of macroscopic type of *Sarcocystis*. Furthermore, Ocular and stage microscopic micrometer measurement (Me Can/ Japanese) was used to calculate the measurement size of microscopic cysts

(Mean ± Standard Deviation) in different muscle tissue of sheep and goats (Hussein et al. 2023b).

$$Microscopic\ factor = \frac{\text{Number of stage micrometer}}{\text{Number of ocular micrometer}}$$
$$= \frac{1mm}{0.10mm} \quad = \frac{1000\mu m}{100\mu m}$$
$$= 10$$
$$= \frac{\text{Number of stage}}{\text{Number of ocular}} \text{ X Factor}$$

Statistical analysis

The collected data was analyzed by using SPSS statistics software (IBM, version 19.0). T- test and chi-square test were applied to find the variation and association between the obtained data form sheep and goats. P>0.05 considered as non-significant, and P<0.05 considered as significant.

RESULTS AND DISCUSSION

During macroscopical examination of slaughtered animals and cutting sections of all collected muscle samples, the macrocystis was only observed in esophageal muscular tubes. The overall prevalence rate of macroscopic cysts in sheep and goats was 1.5% (42/ 2890). The infection rate was 1.2% (28/ 2358) in sheep and 2.6% (14/ 532) in goats, respectively. The lower prevalence rate of macrocystis in both animal species, perhaps related to the lower frequency of the *Sarcocystis* species, insufficient number of definitive hosts in the area which responsible for the formation of this type of cysts or due to the lower probability of food and water being contaminated with sporocysts shed with the feces of feline. This finding was compatible with some other previous reports from different regions. In Iraq/ Baghdad, the prevalence of macrocystis was 4.1% in sheep, 33.6% in goats (Latif et al. 1999) and 34% in goats (Barham et al. 2005). In Turkey, the prevalence rate of macrocystis in sheep from different provinces was ranged from 6.1% to 66% (Okur et al. 1995). In Iran, it was 4.3% (Hosseini et al. 2012) and 17.08% (Farhang-Pajuh et al. 2014), in sheep. In contrast, no macroscopic cysts were recorded in muscle tissues of sheep and goats by Shekarforoush et al. (2005), Shahraki et al. (2018) in Iran, and Zangana & Hussein (2017), in Iraq. Two different types of macroscopic cysts were found along the esophageal muscular tube in sheep and goats. The first type is called thin cyst which appeared as small cylindrical or elongated in shape (rice-grain like), whitish in color & embedded into the muscle tissue of esophagus. The second type is called fat cysts which appeared as milky-white in color, rounded and bulged over the fibrous layer (tunica adventitia) of esophageal wall. The bulged cysts are highly packed with a large number of banana shaped merozoites, while the embedded cysts are less packed with a small quantity of less curved merozoites. The shapes of merozoites in fat (bulged) cysts are more crescent, smaller in size and abundant in quantity with a peripherally located nucleus, whereas or thin cysts (embedded) are less crescent, larger in size and less in quantity with a centrally located nucleus (Fig. 1). The intensity rate of macroscopic cysts (cyst/g or cyst/organ) in esophageal muscle organ was estimated to be 28 esophagi of sheep and 14 esophagi of goats. The total weight of esophageal muscle was 1922.9g in sheep and 986.8g in goats, respectively. The total number of macrocystis (fat cysts and thin cysts) which found in esophageal muscle was 473 cysts in sheep & 331 cysts in goats. The intensity rate of macrocystis in sheep was (4 cyst/ gm or 16.9/organ) and in goats was (3 cysts/g or 23.6/organ), respectively (Table, 1). Statistically, there were significant differences (P<0.05) in the prevalence rate of macrocystis of sarcocystosis between sheep and goats. In this work, macrocystis were only observed in the esophagus muscle tissues. Similar findings were reported by Titilincu et al. (2008) at the rate 26.3%, by Mohammed and Kadihm (2007) at the rate 0.9% and by Beyazit et al. (2007) at the rate 16%. While some authors observed macroscopic cysts in other muscle tissues including skeletal muscle, heart and diaphragm (Latif et al. 1999, Dehaghi et al. 2011). In addition, Barham et al. (2004) found the highest rate of macrocystis 98.9% in esophagus, and the lowest infection rate in skeletal muscle 4.3% and in diaphragm 2.5%. While Hosseini et al. (2012) found the macrocystis in diaphragm of sheep at the rate of 4.3%, respectively. Two types of macrocystis comprising fat cyst (bulged) and thin cyst (embedded) were observed in the esophagus of sheep and goats during examination in this study. Similar study was reported by Farhang-Pajuh et al. (2014). He recorded fat cysts at the rate 27.9% and thin cysts at the rate 8.93%, in straited muscle tissues of sheep. The intensity rate of macroscopic cysts was estimated in this study for the first time through calculating the number of cysts (fat & thin) per gram of muscle tissue (cysts/gram) or by estimating the average number of cysts per organ (cysts/ organ). The severity rate of the intensity rate in sheep and goat maybe related to the ingestion numbers of sporocysts which shed with the feces of felines as the definitive hosts. primally, feline is considered as definitive hosts for macrocystis forming species of *Sarcocystis*. In regard, the species of *Sarcocystis* which suspected in sheep and goats were included, *S. ovifelis* (*S. gigantea)* and *S. medusiformis* in sheep and *S. caprafelis* (*S. moulei*) in goats, respectively. This finding was consistent with studies by Dubey et al. (2015) and Hussein et al. (2025) and Lindsay and Dubey (2020). The overall prevalence rate of microscopic cysts in sheep and goats was 96.5% (220/ 228). The infection rate in sheep was 96.6 % (114/118) and in goats was 96.4% (106/110), respectively. In addition, the infection rate in male was 95.8% (69/72) and 97% (96/99), while in female was 97.83% (45/46) and 91% (10/11) in sheep and goats, respectively (Table, 2). Statistically, there was no significant difference (P>0.05) in the prevalence rate of microcysts between sheep and goats. Similar high prevalence rate of infection was reported by many authors from several countries. It was 96.9% in sheep in Mongola (Fukuyo et al. 2002), 93% in Ethiopia (Woldemeskel and Gebreab 1996) and 76% in Italy (Giannetto 2005). While, low prevalence rate was reported in Algeria 64.4% (Nedjari 2003), In Turkey 58.9% (Ozkayhan et al. 2007), in Iran 57.7% (Oryan et al. 1996) and 47% in Slovakia (Mala and Baranova 1995). The overall infection rate of microcysts in heart and diaphragm was 96.5% (220/228), while in esophagus it was 78.1% (178/228) in sheep and goats. The highest infection rate was similarly recorded in

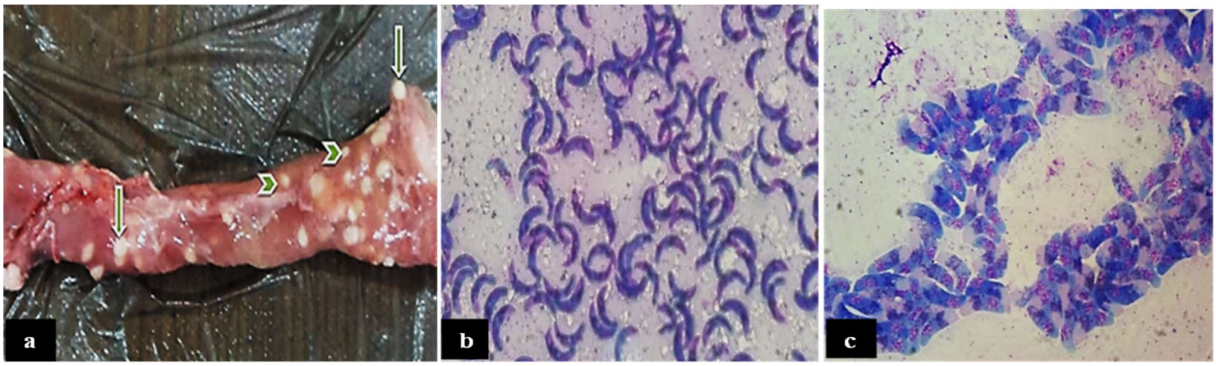

Fig. 1: a. large numbers of macrocystis comprising thin or embedded cysts (arrow head), and bulged or fat cysts (arrow). b. merozoites of embedded cysts (Giemsa stain 100X). c. Merozoites of bulged cysts (Giemsa stain 100 X).

Table 1: The intensity rate of macrocystis (cyst/gm or cyst/esophagus) estimated in Sheep and in Goats.

Animal	Examined Esophagus	Weight/gm	Macrocystis Number	cyst/gm	cysts/ esophagus
Sheep	28	1922.8	473	4	16.9
Goats	14	986.7	331	3	23.6
Total	42	2909.5	804	7	40.5

Table 2: The overall prevalence rate of microscopic cysts of sarcocystosis in male and female of Sheep and Goats.

Sex	Sheep		Goats		Overall	
	Examined Animal	Positive (%)	Examined Animal	Positive (%)	Examined Animal	Positive (%)
Male	72	69 (95.8)	99	96 (97)	171	165 (96.5)
Female	46	45 (97.8)	11	10 (91)	57	55 (96.5)
Overall	118	114 (96.6)	110	106 (96.4)	228	220 (96.5)

the heart and the diaphragm of sheep and goats 96.6% (114/118) & 96.4% (106/110), respectively. While the lowest infection rate was 72.9% (86/118) and 83.6% (92/110) recorded in the esophagus of both animal species, respectively. In addition, the infection rate of mixed cysts (macrocystis and microcystis) is only found in the esophagus organs of sheep and goats. It was 22% (26/118) in sheep and 11.8% (13/110) in goats, respectively (Table, 3). There was no significant difference (P>0.05) in the prevalence rate of microcysts between male and female of sheep and goats. Similar to our investigation, the highest infection rates of 100 & 93.2% were recorded in diaphragm by Fukuyo et al. (2002) and Bahari et al. (2014). Moreover, Lindsay and Dubey (2020), similarly found the highest prevalent rates of infection 22.4% in the diaphragm and the lowest rate 0.23% in the esophagus of goats. Furthermore, a likewise study by El-Morsey et al. (2019) found the highest infection rate 74% in diaphragm, followed by 54.4% in esophagus and 43.3% in tongue, while the lowest rate 38% was detected in the heart in goats in Egypt. In contrast to our results, a study by Dafedar et al. (2008) found the infection rate 69.4% in esophagus, followed by 36.1% in heart and 62.5% in diaphragm of goats in Bangalore province of India. As well as Titilincu et al. (2008) reported the highest prevalence rate, 81.6% in esophagus, followed by 79.9% in heart and 68.8% in diaphragm, in sheep. And, Abdullah (2021) recorded the highest prevalence of microcysts 95% in esophagus and 90% diaphragm, respectively. 99% and 98.9% in esophagus by Barham et al. (2004). The efficiency of the three techniques which were used for detection of microscopic cysts in this study was also evaluated. It found that the peptic digestion technique appeared at the highest efficiency rate of 100% in the detecting of parasitic microcysts (bradyzoites) in all muscle samples of sheep and goats, followed by grinding and squeezing method with Giemsa staining at the rate 97.4% in sheep and 97.3% in goats, respectively. While the muscle squash method has the lowest efficiency in the detection of microcysts at the rate 86.7% in sheep and 85.7% in goats, respectively (Table, 4). Statistically, no significant differences (P>0.05) were observed in the detection of the parasitic infection by the three techniques between sheep and goats. Similar data were recorded by several authors from different regions. In Iraq, the highest prevalence rate 100% of sarcocystosis was recorded by both pepsin digestion technique and mincing & squeezing method, and 95% by muscle squash method in sheep and goats in Duhok (Zanagana and Hussein 2017). In goat, the prevalence was 93.22% by Pepsin digestion and Squeezing method in Babylon (Mohammed & Kadihm, 2007). The overall prevalence rate of microcysts was 97% in sheep & 97.4% in goats by Latif et al. (1999). They also found the peptic digestion with the highest efficiency rate of 93.3%, followed squeezing 81.3% and muscle squash 81.2%, in Baghdad. Furthermore, the prevalence rate of microcysts was 97.4% in goats by peptic digestion and 89.7% by muscle squash method in Sulaymaniyah (Barham et al. 2004). In Turkey, it was 100% in male and female of sheep (Okur et al. 1995). In Iran, it was 100% in goats by peptic digestion method with no significant differences (P>0.05) between male and female. 100% in sheep and cattle in the United States (Fayer et al., 2015). 100% by pepsin digestion in sheep (Zangana and Hussein, 2017). In Egypt, the prevalence rate in goat was 79.4% by muscle squash method (El-Morsey et al. 2019). The overall measurement size of free microcysts in sheep was 13.3±1.7µm in length & 7.4±0.7µm in width, and in goats was 11.4±1.3µm in length & 6.7±0.7µm in width,

respectively. The largest average size of microcysts which found in the diaphragm of sheep was 14.4±2.2 x 6.8±0.8µm and the heart of goats was 12.5±1.6 x 7±0.7µm, while the smallest size was found in the esophagus of both animal species. It was 12.4±1µm in length & 8.8±0.6µm in width in sheep, and 10.2±1µm in length & 6.2±0.6µm in width in goats, respectively. No significant difference (P>0.05) was observed between the average size of cysts in all muscle tissues in both animals. Different shapes of tissue cysts were observed by muscle squash method and mincing & squeezing technique with Giemsa staining in different muscle tissue of both animal species including spindle, elongated, rounded, spiral and cylindrical. While, by peptic digestion method, the cysts were raptured and hundreds of free bananas shaped bradyzoites were released (Fig. 2). The variation in the size of microcysts is perhaps due to the maturation duration period of the bradyzoites or developmental stage of cysts in the tissue. The variety in the intensity rate of microcysts in all muscle tissues is related to the capability of the protozoan parasite to develop and multiply in muscle tissues. The intensity rate of microscopic cysts (cyst/ field of microscope) from examined tissues stained with Giemsa is the newly achieved technique in this study. The overall intensity rate of microcysts was 32.4 cyst/ field (5833/180) in sheep and 16.8 cyst /field (3015/180) in goats, respectively. In sheep, the highest intensity rate of 52.76 cyst/ field (3166/60) was found in diaphragmatic tissues and the lowest rate 14.5 cyst/ field (870/60) was found in esophagus, while in heart the intensity rate was 29.9 cyst/ field (1797/60). In goats, the highest intensity rate was found in the heart muscle 20.2 cyst/ field (1212/60), and the lowest rate was found in esophagus 13.9 cyst/ field (835/60), while the intensity rate in diaphragm was 16.1 cyst/ field (968/60) (Table**,** 5). Statistically, significant difference (P<0.05) of the parasitic intensity rate was found between sheep and goats. Histopathological features of shape, size; intensity rate & wall thickness of microscopic cysts in muscle tissues of sheep and goats were also recorded in this study. The histopathological examination through staining of muscle tissues with H&E stain, PAS-stain and Giemsa stain shows different shapes (oval and spindle) and sizes (small & large) of cysts packed with numerous numbers of bradyzoites (Fig. 3). The typical shape of the tissue cyst has many compartments (septa) which are fully packed with bradyzoites. These septa have a finger-like protrusion starting from the cell wall to the inside of the cyst. Most of the cyst walls in goat muscle tissues have appeared with a typical radial striation. The histopathological reactions were closely similar in all muscle tissues. They vary from mild, to moderate granulocytosis (eosinophilia) and mononuclear cells infiltration surrounding the intact microcysts to necrotizing and degeneration of muscle fibers (myositis). Some tissue cysts were raptured and bradyzoites were released (Fig. 4). Two different types of microcysts wall (thin and thick) in both animals are observed through

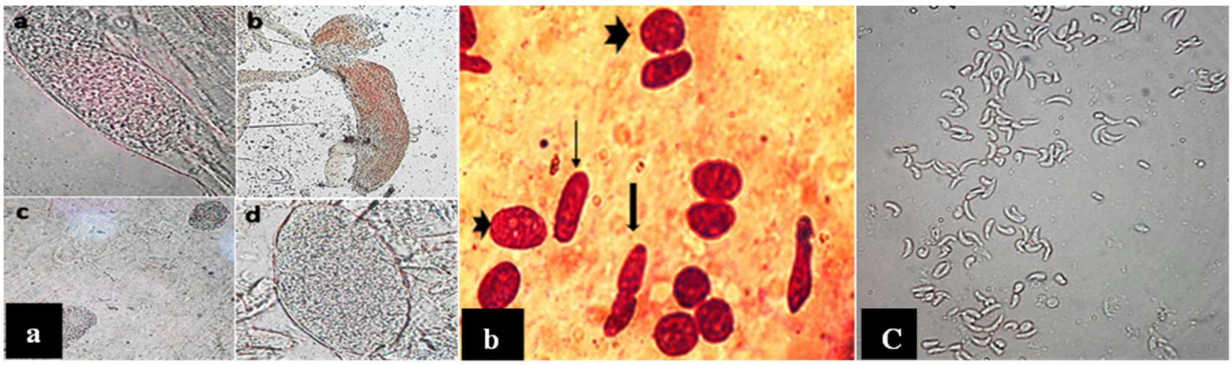

Fig. 2: a. Muscle squash method (40X).(a: spindle-like microcysts (40X). b: spiral microcysts (40X). c: Oval-shape of microcyst (40X). d: rounded-shape of microcysts (40X)). b. microcysts in diaphragm of sheep (Giemsa stain 100X), cylindrical-cyst (thin arrow), spindle-cyst (Thick arrow), rounded-cyst (big arrow head), oval-cyst (small arrow head). c. banana shaped bradyzoites from distracted cyst after pepsin digestion (wet mounting 40X).

Table 3: The percentage rate of both types of cysts (marcocystis and microcysts) in different muscle tissues of both animal species.

Animal	Esophagus				Diaphragm			Heart		
	No.	No. of Micro cysts %	No. of Macro cysts %	No. of Mixed cysts %	No. of Micro cysts %	No. of Macro cysts %	No. of Mixed Cysts %	No. of Micro cysts %	No. of Macro cysts %	No. of Mixed cysts %
Sheep	118	86 72.9	2 1.8	26 22	114 96.6	0	0	114 96.6	0	0
Goats	110	92 83.6	1 0.9	13 11.8	106 96.4	0	0	106 96.4	0	0
Total	228	178 78.1	3 1.4	38 16.7	220 96.5	0	0	220 96.5	0	0

Table 4: The efficiency rate of the methods used for the detection of microcyst in different muscle tissues of Sheep and Goats.

Animal	Muscle squash method			Mincing &Squeezing with Giemsa staining			Peptic Digestion		
	Examined Number	positive	%	Examined Number	positive	%	Examined number	positive	%
Sheep	15	13	86.7	77	75	97.4	26	26	100
Goats	14	12	85.7	75	73	97.3	21	21	100
Overall	29	25	86.2	152	148	97.4	47	47	100

Table 5: The intensity rate of microcysts in muscle tissues of esophagus, diaphragm & heart of Sheep and Goats.

Animal	Esophagus	Diaphragm	Heart	Overall	
	No. of Cysts	No. of Cysts	No. of Cysts	No. of cysts	No. of fields
Sheep	870	3166	1797	5833	180
	14.5/field	52.76/field	29.95/field	32.4/ field	
Goats	835	968	1212	3015	180
	13.9/field	16.1/field	20.2/field	16.8/field	

microscopical examinations. Generally, the walls of cysts in sheep muscle tissues were thinner than of cyst's wall in goats (Fig.5). The intensity rate of histopathological stained tissue cysts was estimated by calculating number of cysts/ examined field. The highest intensity rate of cysts was 4.3 cysts/field, and 3.1 cysts/ field was detected in heart muscle tissue of sheep and goats, followed by esophagus 3.25 cysts/ field in sheep and 2.7 cysts/ field in goats. While the lowest intensity rate was found in the diaphragm 2.5 cysts/ field in sheep and 2.3 cysts/field in goats (Table, 6). The average size (Mean ± Standard Deviation) of round and spindle shaped microcyst and the thickness of their cell walls were measured from muscle tissues of sheep and goats. The largest average size of spindle shaped cyst (290±89.7 x 76.1±10 µm) and round shaped cyst (88.8±10.3µm) was detected in the esophagus of goats. In heart, the spindle size of cyst was (123.3±15.9 x 42.6±6.2µm) and the round cyst size was (59.2±13.7µm). While the smallest spindle shaped size (108±20.6 x 56±9.1µm) and round shaped size cysts (54.2±4.5 µm) was detected in diaphragm. In sheep, the largest spindle and rounded shaped size of cyst (127.2±18.9 x 53.3±5.4µm) and (74.4±7.5µm) was found in esophagus. In heart, the spindle shaped size was (106.8±4.2 x 41.4±5.3µm) and round shaped size was (39.1±4.2µm). while smallest size of spindle (92.4± 4.9 x 34.8±0.7µm) and rounded shaped cysts (43.8±4.5µm) was recorded in diaphragm (Table, 7). similar measurement size of spindle shaped cysts (225 – 431.3µm x 51.4 – 82.3µm) was found in esophagus and rounded shaped cysts (51.4 – 82.3µm) was recorded in heart of sheep (Nermean et al. 2018). In contrast, Abdel-Baki et al. (2009), reported different sizes of immature and mature microcysts of *Sarcocystis* in esophagus muscle tissues. The mature cysts size was measured 670 - 800µm in length & 170 – 200µm in width. The thickness of wall that surrounded the microcysts in goats muscle tissue was generally thicker than in sheep. In goats, the thickest wall of spindle cysts was 8.4±1.2µm and the thinnest wall of it was 3.9± 0.7µm. While in sheep, the thickest wall of spindle cyst was 6±0.7µm and the thinnest wall of it was (2.7±0.3µm). In goats, the thickest wall of rounded microcyst was 7.2±0.6µm and the thinnest wall of it was 4.4±0.3µm. In sheep, the thickest wall of round cyst was 5.4±0.9µm and the thinnest wall of it was 2.6±0.2µm. There were no significant differences ($P>0.05$) found in the measurement size and the intensity rate of both types of microcysts between sheep and goats (Table, 7 & 8). The histopathological investigation of muscle tissues of esophagus, diaphragm and heart showed different shapes, size, pathological reactions and intensity rate of the microcysts in both intermediate hosts, in this study. Morphologically, the parasitic cysts in different muscle tissues are relatively similar. The microcysts have generally appeared round and elongated spindle-like in shape with thick or thin walls as well as different sizes between muscle fibers in sheep and goats (Hussein et al. 2023). Commonly, the wall and size of microcysts which invade muscle tissue of goats was thicker than sheep and they have a characteristic of radically striated like protrusions toward the inside of the cysts. Similar feature was reported by Beyazit et al. (2007), they morphologically differentiated two types of microcysts between muscle tissues by depending on the thickness of cysts comprising thin or thick walls. In addition, a study by Morsey et al. (2011), found microcysts with thin-walled cysts which regarded to *S. ovicanis* (*S. tenella*) species and thick-walled types regarded to S. *arieticanis* species of *Sarcocystis*. Furthermore, Nourani et al. (2010) and Hussein et al. (2025) differentiated thin-walled *S. cruzi* from thick-walled *S. hirsuta* or *S. hominis* from cattle in Iran and in Iraq. In addition, Abdullah (2021), suspected *S. tenella* and *S.cruzi* from microcysts having thin-walled type, and *S. arieticanis*, *S. bovofelis* and *S. hominis* from microcysts having thick-walled type. In regards, two species of *Sarcocystis* were suspected in each animal species in this study, including *S. tenella* (*S. ovicanis*) and *S. arietcanis* with thin-walled cyst in sheep and *S. capracanis & S. hircicanis* with thick-walled cysts in goats, respectively. However, the difference between species of *Sarcocystis* can't be distinguished by light microscope, while the identification of *Sarcocystis* spp. can be characterized by transmission electron microscope and molecular studies (Gjerde 2016). In this study, mild to moderate tissue reactions against microcystic invasion were observed in all muscle tissues. In mild reaction, there were a few numbers of inflammatory cells commonly granulocytes (eosinophils) and mononuclear cells infiltrated around the cysts. While, in moderate response, there was some necrosis and degeneration of myofibrils with moderate infiltration of eosinophils and monocytes surrounding the area of necrosis with mild eosinophilic myositis. Some microcysts wall were raptured and free bradyzoites escaped surrounding the muscles fibers. Similar pathological features were observed by Beyazit et al. (2007), El-Morsey et al. (2019) and Abdullah (2021). In addition, through an experimental study by Dubey et al. (2015), there was initial hemorrhagic stage of tissue followed by invasion of first generation merozoites which perhaps due to early severe inflammatory response. Marandykina-Prakienė et al. (2022) found marked mononuclear cells inflammation in central nervous system, heart, tongue and liver, but tissue of skeletal muscle, lung and kidney were slightly affected. Hussein et al. (2025b) observed that the pathological lesion was reliant on the ingestion of sporocyst's number and the tissue necrosis. They also found distraction of vascular endothelium, rapture of parasitic cysts and hemorrhage of the skeletal muscle and heart tissue in acute infection.

Table 6: The intensity rate of *Sarcocystis* microcyst in different muscle tissues of Sheep and Goats by histopathological technique.

	Esophagus		Diaphragm		Heart	
Animal	Fields	No. of cysts	Fields	No. of cysts	Fields	No. of cysts
Sheep	20	65 (3.25/field)	20	50 (2.5/field)	20	86 (4.3/field)
Goats	20	54 (2.7/field)	20	46 (2.3/field)	20	62 (3.1/field)

Table 7: The measurement size and thickness of cysts wall with mean & standard deviation (M ± S.D) of microcyst in different muscle tissues in Goats.

Muscle Organs	Goats				
	Ranges (Lengths & Widths) & Averages (M ± S.D)				
	Spindle-Cysts			Rounded-Cysts	
	Lengths	Widths	Cyst's wall	Lengths	Cyst's wall
Esophagus	200.4-379.8 (290.1±89.7)	66.1-86.1 (76.1±10)	4.3-7.1 (5.7±1.4)	78.5-99.1 (88.8±10.3)	6.6-7.8 (7.2±0.6)
Diaphragm	87.4-129 (108±20.6)	46.9-65.1 (56±9.1)	7.2-9.6 (8.4±1.2)	49.7-58.7 (54.2±4.5)	5.9-7.7 (6.8±0.9)
Heart	107.4-139.2 (123.3±15.9)	36.4-48.8 (42.6±6.2)	3.2-4.6 (3.9±0.7)	45.5-72.9 (59.2±13.7)	4.1±4.7 (4.4±0.3)

Table 8: The measurement size and thickness of cyst wall with mean & standard deviation (M ± S.D) of microcyst in different muscle tissues in Sheep.

Muscle Organs	Sheep				
	Ranges (Lengths & Widths) & Averages (M ± S.D)				
	Spindle-Cysts			Rounded-Cysts	
	Lengths	Widths	Cyst's wall	Length	Cyst's wall
Esophagus	108.3-146.1 (127.2±18.9)	58.7-58.7 (53.3±5.4)	4.1-5.5 (4.8±0.5)	66.9-81.9 (74.4±7.5)	3.76-5.36 (4.56±0.8)
Diaphragm	87.5-97.3 (92.4±4.9)	34.1-35.5 (34.8±0.7)	5.3-6.7 (6±0.7)	39.3-48.3 (43.8±4.5)	4.5-6.3 (5.4±0.9)
Heart	102.6-111 (106.8±4.2)	36.1±46.7 (41.4±5.3)	2.4-3 (2.7±0.3)	34.9-43.3 (39.1±4.2)	2.4-2.8 (2.6±0.2)

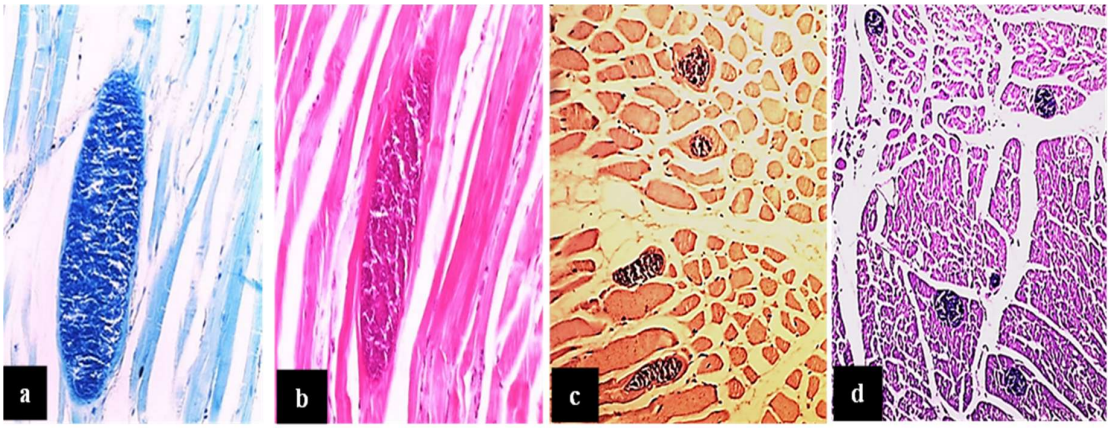

Fig. 3: Histopathological examination. a & b. Spindle-shaped microcysts in the esophagus (a. Giemsa stain 20X and b. H &E stain 20X.) c. Spindle and rounded cysts of microcyst in diaphragm (H& E stain 10X). d. rounded microcysts in heart (PAS stain 10X).

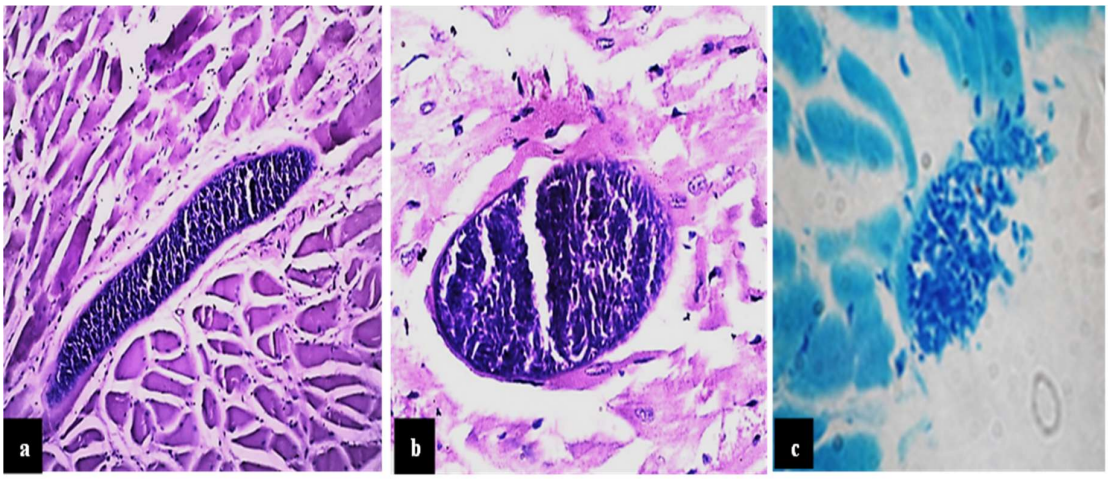

Fig. 4: Histopathological sections illustrate. a. Intact microcyst infiltrated by inflammatory cells (PAS stain 20X). b. Microcyst with muscle degeneration and eosinophilic infiltration (PAS stain 40X). c. Distracted cyst's wall of microcysts and release of free bradyzoites (Giemsa stain 40X); bradyzoites (Giemsa stain 40X).

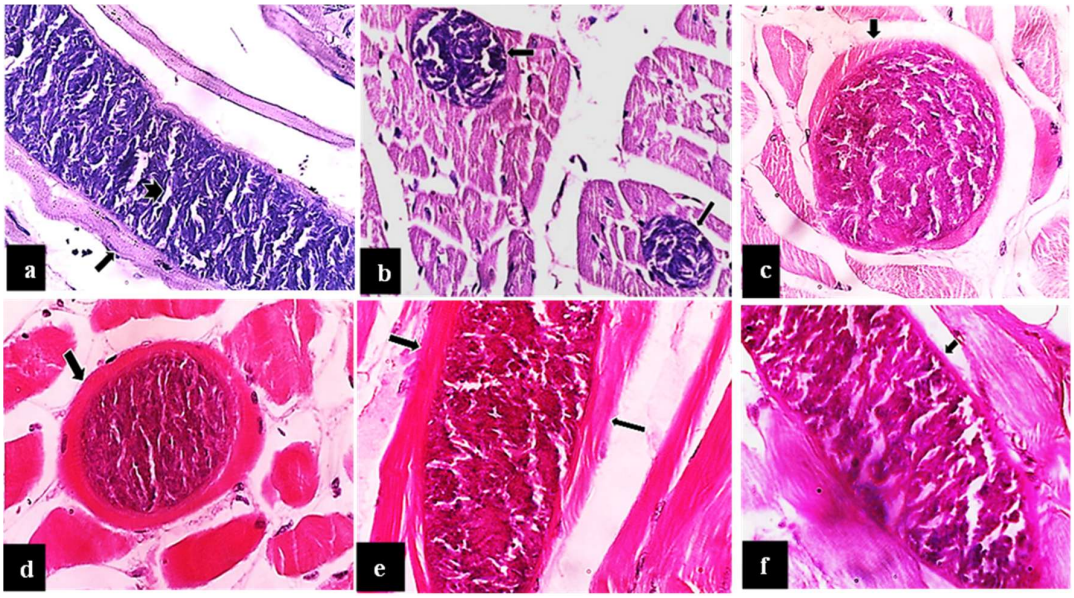

Fig. 5: Histopathological sections of mature microcysts in different muscle tissues. a. Sepat or finger-like protrusion (Head arrow), thick cyst wall (Arrow) in goats, (PAS stain, 40X). b. Thin microcysts wall in sheep, (PAS stain, 20X) c. Radial striation of cyst in goats (H&E stain, 40X). d. thick cyst wall of cysts in goats (H&E stain, 40X). e. Radial striation thick cyst wall in goats (PAS stain, 40X). f. thin cyst wall in sheep (H&E, 40X).

Conclusions

Two types of *Sarcocystis* including macrocystis and microcysts which present in the striated and non-striated muscle tissues are recorded in this study. Microscopic type of *Sarcocystis* is more prevalent than macroscopic type. Two types of macrocystis including, the thin or embedded cysts and fat or bulged cysts were found in esophagus muscle tissue of sheep & goats. In sheep, the macrocystis was regarded to *S. ovifelis* and *S. medusiformis*, while in goats, it was regarded to *S. capracanis* with feline as their definitive host. For the first time, the estimation of intensity rate of macrocystis that formulated by calculating the number of cysts/ gram of tissue or cysts/ organ, and intensity rate of microcysts which formulated by calculating the number of cysts/ microscopic field after mincing and squeezing preparation and staining with 5% of Giemsa stain was performed in this work. Spindle and rounded shaped microcysts with different size and wall thickness were observed through histopathological examination in muscle tissues of sheep and goats. Wall thickness of microcysts in sheep was smaller than in goats. Through morphological analysis of wall thickness of microcysts, two species of *Sarcosystis* were suspected in each host including *S. tenella (S. ovicanis)* and *S. arietcanis* with thin-walled cyst, in sheep and *S. capracanis & S. hircicanis* with thick-walled cysts in goats, respectively. Mild to moderate pathological reactions with inflammatory cells infiltration and degeneration of myofibrils surrounding the microcysts were observed in different muscle tissues of the both intermediate hosts.

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