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Prevalence of *Sarcocystis* in slaughtered sheep carcasses in AL-bieda, Libya

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Abstract

Sarcocystis is an obligatory intracellular protozoan parasite, which can infect human and animals. Sheep are intermediate hosts for *Sarcocysitis* species. Despite the importance of worldwide sheep production, little is known about the prevalence of *Sarcocystis* in domestic sheep (Barbary breed) in Libya. The main aim of the present study is to determine the prevalence of *Sarcocystis* in sheep in Al-beida city, Libya, as well as to identify parasite based on macro and microcysts examination. Thirty five sheep (25 male and 10 female) slaughtered at local abattoirs in Al-beida city. Muscle samples were collected from heart, esophagus and diaphragm (35 each) of sheep carcasses and examined by digestion method igesting pepsin and Hcl for bradyzoites observation in the organs by light microscope. The results showed that, microcysts were more prevalent than macrocysts which were detected in only one esophagus sample in agedmore than one year age. The digestion technique revealed positive results in all samples taken from heart, diaphragm and esophagus (100%, 97% and 94%) respectively. There were significant differences between different ages, while the prevalence of *Sarcocystis* was not influenced by sex and seasonal variations. It could be concluded that prevalence of *Sarcocystis*is significantly high in Al-beida city.

Keywords: *Sarcocystis spp., sheep, macrocysts, microcysts.*

Introduction

Sarcocystis is an obligatory intracellular protozoan parasite, which belongs to the phylum apicomplexan and infects a wide range of livestock. *Sarcocystis* species require two obligatory hosts to complete their life cycle, carnivores as definitive hosts and herbivores as intermediate hosts. The intermediate hosts become infective by either ingestion of sporocysts in the environment or form muscular cysts. Definitive hosts are infected by ingested the muscular cysts containing bradyzoites.Lesions caused by *Sarcocystis* cancause economic impact on the production of domestic animals, particularly in sheep, goats, cattle, camels and buffaloes (**Gopal *et al*., 2016; Mirzaei-Dehaghi *et al*., 2013**). Humans are also infected with these parasites as either definitive or intermediate hosts where they inhabit the muscles and intestinal tract. Humans acquire infection by ingestion cysts through eating raw or undercooked infected meat or meat-products **(Motamedi *et al*., 2011; Rahdar & Salehi., 2011).**

More than 150 species of *Sarcocystis* parasitize humans and animals, from which, so far, sheep are infected by four species*, S. tenella* (*S. ovicanis*) and *S. arieticanis* considered to be pathogenic and mainly transmitted by dogs. Whereas *S. gigantean* (*S. ovifelis*) and *S. Medusiformis* are non-pathogenic, which mainly can be transmitted by cats (**Pejman *et al.*, 2014; Bittencourt *et al*., 2016**). These species are responsible for formation of micro and

macro cysts in different organs of the body including skeletal, cardiac muscle, esophagus and diaphragm, with high prevalence in sheep **(Damboriarena *et al*., 2016)**. Studies in different regions around the world demonstrated that the prevalence of *Sarcocystis* infection in slaughtered cattle and sheep are between 70% to 100% **(Bahari *et al*., 2014; Pereira & Bermejo, 1988)**. Additionally, a study in western Libya revealed that the prevalence of *Sarcocystis* in sheep was 86.1% **(Elhussein, 2001)**. Sarcocystis lesions are found in the muscle of sheep at the abattoir. These small white cysts resemble grains of rice and commonly affect the esophagus, tongue, diaphragm and skeletal muscle. The aim of the present study was to determine the prevalence of *Sarcocystis* sp. for the first time in the sheep carcasses in AL-beida city in eastern Libya.

Materials and Methods

Post mortem examination (Macroscopic examination):

All carcasses were apparently inspected (P.M examination) at abattoir for the presence of macrocysts of *Sarcocystis*, with focusing on the muscles of heart, diaphragm and esophagus. Tissues that found infected with grossly macrocysts were collected and transported to the laboratory for further morphological examination.

Collection of Samples

Fresh muscle samples of heart, diaphragm and esophagus were collected randomly from35 carcasses(25 male and 10 female) of sheep slaughtered in local abattoirs in AL-beida city between September to December (2016). All of the collected samples were transported to the laboratory within two hours of collection for detection of microcysts of Sarcocystis.

Microscopic examination

Samples of heart, diaphragm and esophageal muscles were examined for the presence of intramuscular *Sarcocystis* by using digestion technique.

Peptic digestion method

Ten gram of each tissue were minced by blender and digested for 20 minutes at 40-45°C in 50 ml of digestion medium (37% HCL and 0.5% pepsin). The digests were filtered through a fine mesh sieve (mesh aperture 0.5mm) into a beaker, and then centrifuged at 2000 rpm for 5 minutes. The supernatant layer was discarded and the sediment was suspended in 0.5ml of 0.85%w/v normal saline. Approximately, 0.05 ml of suspension was examined for the presence of *Sarcocystis* bradyzoites under the light microscope at 40-x magnification **(Collins, 1980).** In addition, further drop from the same solution was spread on slides, fixed, stained with 1% Giemsa's stain and examined microscopically as well **(Farhang-Pajuh *et al*., 2014).**

Statistical analysis

Data were analyzed by SPSS software (version 16) and Chi square (x^2) test analysis. The significance level was $P<0.05$.

Results

A total of 35 slaughtered sheep carcasses were examined for the presences of macro or microcysts of *Sarcocystis* and all the samples were tested by peptic digestion method. The results showed that the highest prevalence of microcysts were recorded in the heart 100% (35/35) followed by diaphragm 97.1% (34\35) while, the lowest prevalence were determined in the esophagus 94.3% (33\35). In addition, all samples were infected with bradyzoites of *Sarcocystis* (Table 1 and Fig 1 a and b). Furthermore, macrocyst of *Sarcocystis* was observed in the muscular tissue of one oesophagus 2.9% (1 \35),as detected with a naked eye in a female sheep within 3 years old. Two types of cysts were determined and distributed randomly throughout the oesophagus (Table 1). The cysts were identified as fat and thin cysts and they were milky- white in colored. The fat cysts appeared to be large, thick and round or oval in shape with a length of 20 mm and width of 0.8 mm (Fig. 2 a). Whereas the thin cyst was small, slender and less frequent than the fat

Table (1). Detection of micro and macrocysts of *Sarcocystis* in different muscle tissues.

	Heart		Diaphragm		Esophagus	
	Micro	**Macro**	**Micro**	**Macro**	**Micro**	**Macro**
Samples	35		35		35	
Detection	35	0	34	0	33	1
%	100	0	97.1	0	94.3	2.9

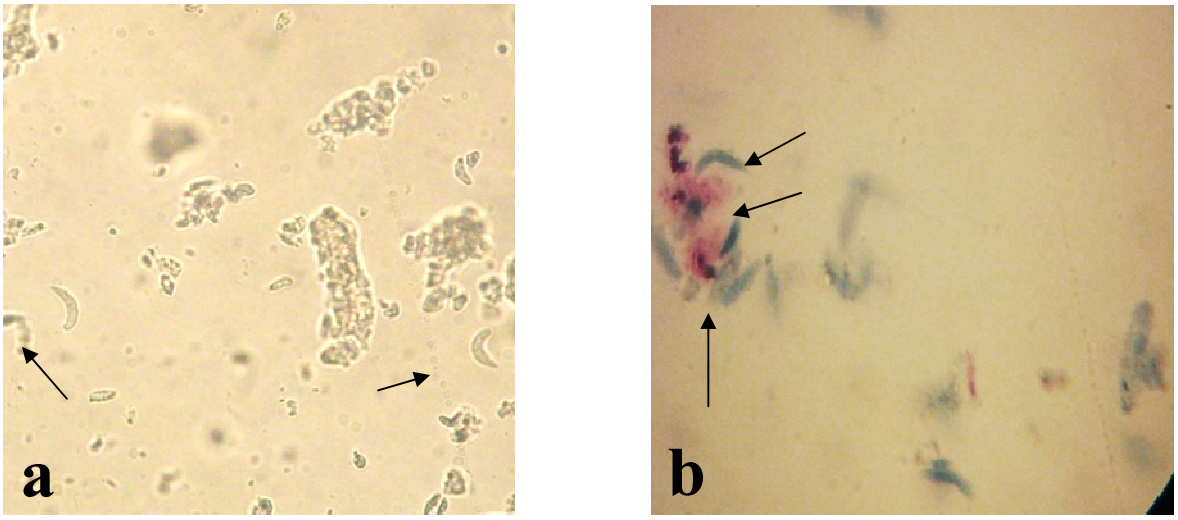

Figure (1). The bradyzoites of *Sarcocystis* sp. (arrows) by peptic digestion method (X40). a) without Giemsa's stain, b) with Giemsa's stain.

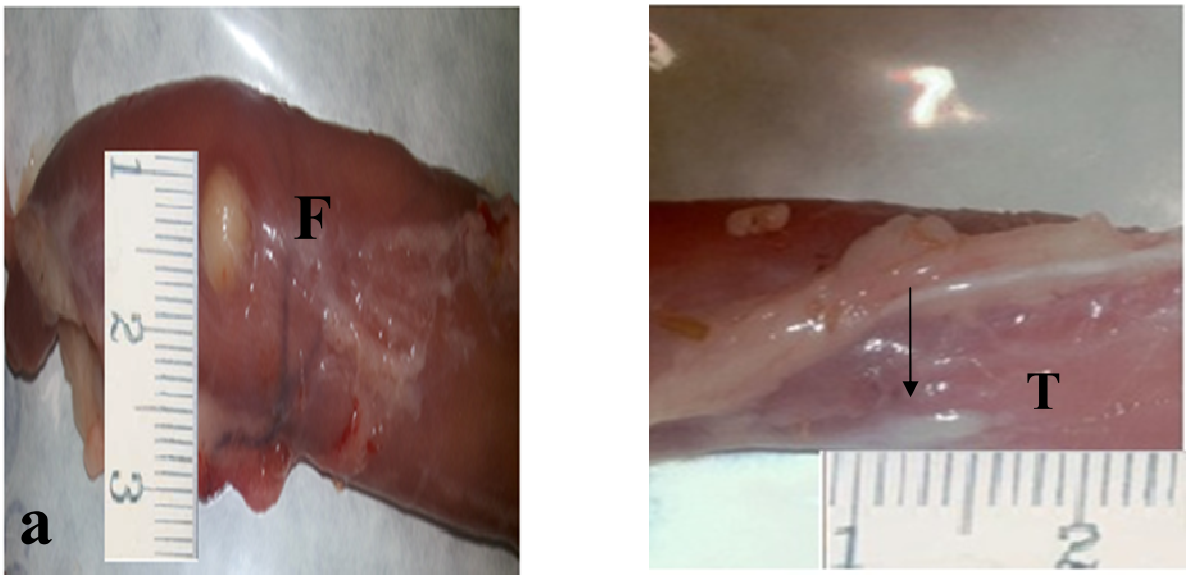

Figure (2). (a) Fatmacrocyst of *Sarcocystis* (F) 20x0.8 mm and **(b)** Thinmacrocyst of *Sarcocystis* (T) 14x0.3mm in esophagus of sheep.

In terms of age, the overall prevalence of microcysts was found to be 100% (35\35) (Table 2). Microscopically, out of 35sheep under one year old, 23 (65.7%) were found to be infected, whereas12 (34.3%) of sheep over one year old were detected in examined animals. Therefore, there were a significant differences in the infection rates of *Sarcocystis* with the respective to different ages ($p<0.05$).

Table (2). Prevalence of microcysts of *Sarcocystis* in different age of sheep carcasses:

Age in years	No. of examined carcasses	No. of infected carcasses	% of infection
<1	23	23	65.7
>1	12	12	34.3*
Total	35	35	100

***** indicates significant differences at (p <0.05).

In males and females, the prevalence of microcysts were 97.3% (73\75) and 96.7% (29\30), respectively. Therefore, there were no significant differences in the prevalence rates of microcysts in different sex of examined samples (p> 0.05) (Table 3).

Table (3). Prevalence microcysts of *Sarcocystis* in different sex of sheep.

Sex	No. of examined samples	No. of detected samples	% of infection
Male	75	73	97.3
Female	30	29	96.7

The severity of microcysts prevalence was recorded in three months as follow: the prevalence of infection was high in October, November and December (100%, 97.8%and 90.5%), respectively. There was no significant difference in the infection rates between months (P>0.05). The results were described in table (4).

Table (4). Monthly prevalence microcysts of *Sarcocystis* in sheep.

Months of examination	No. of examined samples	No. of detected samples	% of infection
October	39	39	100
November	45	44	97.8
December	21	19	90.5

Discussion

Sarcocystis is a wide range distributed and they can cause enormous economic losses in livestock (**Gopal *et al*., 2016**). Many animals can be infected with one or more species of *Sarcocystis* which form macroscopic and microscopic cysts. They were presented either in single or mixed infections as have been shown by many investigations **(Savini *et al*., 1992; Gopal *et al*., 2016).**

This investigation showed that both two types of cysts, microcysts and macrocysts, were detected in examined samples. In addition, all tissue samples have a high frequency of microcysts of *Sarcocystis* in 35 (100%) of slaughtered sheep in Al-beida city, Libya. The results were in the same line with other studies that have been reported a high prevalence of *Sarcocystis* sp. in sheep in various countries. These countries are described as follows: western Libya (86.1%), the United States (100%), France (94.8%), Ethiopia (93%), Slovaki (87.6%), Turkey (90%), Iraq (97%), Mongolia (96.9%) (**Elhussein, 2001; Mirzaei-Dehaghi *et al*., 2013**). Also, the current study showed that microcysts were frequently determined in examined organs by using peptic digestion method. The investigators estimated that the existence of one population of microcysts were associated with *S. tenella*or *S. arieticanis*. According to previous investigations, these *Sarcocystis* sp. are responsible for forming microcystsin different organs and were isolated from different organs of sheep (**Da Silva and Langoni, 2009; Dalimi *et al*., 2008; Bahari *et al*., 2014**). Sheep are infected with microcysts when they ingests porocysts or oocysts that contaminated grass by faeces of dogs, definitive host **(Savini *et al*., 1992).**

In addition, in the present study, two forms of macrocysts were clearly observed in the oesophagus of sheep and they were distinguished as *S. Gigantean* and *S. Medusiformis* based on size, shape and location. Similar observations were described by **Collins *et al.* (1976, 1979)** in New Zealand. Also, the current results noticed that fat cysts which are identified as *S. gigantean*, were thick, round or oval in shape and seemed to grow in length and width. Whereas, the cyst of *S. Medusiformis* was thin, slim, slender and growing widely in length.

Moreover, both of the cysts were found in the oesophagus tissue and this finding was in agreement with previous studies revealed that fat macrocysts are predominantly found in the oesophagus, larynx, and lingual muscles **(Oryan *et al*., 1996; Damboriarena *et al*., 2016).** However, this observation was in contrast with other investigations, which reported that fat cysts were commonly determined in diaphragm, abdomen, and skeletal muscles **(Heckeroth and Tenter, 1998; Farhang-Pajuh *et al*., 2014).** Although, macrocysts of *Sarcocystis* are almost non-pathogenic species in sheep, they are responsible for economic losses due to the complete or partial rejection of the animal carcasses at slaughter houses (**Farhang-Pajuh *et al*., 2014**). While, microcysts of *Sarcocystis* are pathogenic species and they can cause loss of weight, reduced milk production, anemia and abortion. They lead to death in severe infection **(Heckeroth and Tenter, 1998).**

Furthermore, The result of the present study indicated that the lack of macroscopic cysts (2.9%) compared with the prevalence of microcysts (100%) maybe due to the fact that contact between sheep and sporocysts from canine faeces (definitive hosts for microscopic cysts) in Al-beida's pasture is greater than between sheep and cats (definitive hosts for macroscopic cysts). In addition, there are no control or eradication schemes in many countries including Libya **(Savini *et al*., 1992; Mirzaei-Dehaghi *et al*., 2013; Elhussien, 2001).**

Our results showed that heart muscles was the most infected site (100%) followed by diaphragm (97%), then oesophagus (94%). Studies carried out by various authors from different countries have reported that heart, diaphragm and oesophagus were preferred to be examined for *Sarcocystis* infection in the intermediate hosts **(Mirzaei-Dehaghi *et al*., 2013; Daryani *et al*., 2006).** However**, Dayashanker**

(1991) reported the diaphragm as the most infected tissue in the body followed by oesophagus, whereas other investigations revealed that the oesophagus is the most commonly affected organ than any other parts of the body **(Mirzaei and Rezaei, 2014; Damboriarena *et al*., 2016).** The differences in the distribution of *Sarcocystis* sp. in the internal organs of the examined hosts may be due to the oocysts contamination and differences in the ecological and nutritional status of the hosts. These factors can lead to variations in the level of immunity response against these parasites and the infection as well **(Shazly, 2000; Mehlhorn, 2008; Abdel-Ghaffar *et al*., 2009).**

Regarding the age of the examined sheep, results in the present work indicated that the differences in the prevalence between two age groups were significant (p<0.05) in sheep under 1 year old (65.7%). Similar results were reported in lambs in different countries for instance, under 6 months(22.6 %) in Austria **(Egger, 1994),** under 3 months (58.33 %) and (79.05 %) in those between 3 and 12 months of age in Spain (Martinez *et al.*, 1989). In a study performed in Turkey, they found that the prevalence of microcystswas ranged from 34 % to 100 % in sheep under 1 year old **(Beyazit *et al*., 2007).**

With regard to the sex of the examined sheep in the current investigation observed that there were no significant differences in the prevalence of microcysts between the examined male and female sheep. However, the higher overall prevalence among females may be reflection of the age influence. Based on the simple fact that female sheep are slaughtered at an older age (3 years and over) than males (6 months – 1 year) and the governmental policy do not allow the slaughtering of females under 3 years of age. Other studies also revealed that *Sarcocystis* sp. were more frequently reported in adult sheep over 2 years of age than in younger sheep **(Beyazit *et al*., 2007; Britt and Baker, 1990; Abo-Shehada, 1996).**

Moreover, in the present study, the prevalence of microcysts of *sarcocystis* was not influenced by seasonal variations, which might be associated with the short life-cycle of *Sarcocystis* sp. and possibly the rapid rate of maturation of microcysts incomparison to macrocysts. Additionally, our investigation confirmed that there was a high rate of natural infection in the muscles of the examined hosts (sheep). Sheep are frequently exposed to infection due to their close contact with dogs, cats and even wild animals which they act as final hosts for these protozoa. To the authors' knowledge, this study is the first confirmation of the prevalence of *Sarcocystis* spp. in Al-beida city in the eastern region of Libya by using digestive and morphologic methods.

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