

## MICROSCOPICAL AND SEROLOGICAL STUDIES WITH ULTRASTRUCTURE DESCRIPTION OF *SARCOCYSTIS SPECIES* IN SHEEP IN ASSIUT

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### ABSTRACT

This study was conducted to determine the infection rate of *Sarcocystis species* in sheep in Assiut. A total of 100 slaughtered sheep examined for *Sarcocystis*, no macroscopic cysts were observed, 93% (93/100) were positive by microscopical examination and 28% (28/100) were positive by using Agar gel diffusion test. The infection rate of *Sarcocystis* had very high significant differences between microscopic examination and Agar gel diffusion test. High statistical significant effect was found on the infection rate of *Sarcocystis* in different examined muscles of sheep. The highest infection rate was recorded in oesophagus (71%), followed by diaphragm (65%), tongue (58%), skeletal muscles (53%) and heart muscles (43%). The infection rate of *Sarcocystis* in males was 95.2% (40/42) while in females were 91.4% (53/58) by microscopical examination. Higher infection rate of *Sarcocystis* were detected in sheep 6 months - 2 years age 95% (38/40) than that of sheep between 2-4 years old 92.7% (38/41) and those equal or older than 4 years 89.5% (17/19). No significant difference between infection and age groups or sex of animals was observed. The use of transmission electron microscope (TEM) allowed the identification of *S. tenella* and *S. arieticanis* in sheep in Assiut. Examination of *S. tenella* cyst wall with TEM showed palisade-like villar protrusions while, examination of *S. arieticanis* cyst wall showed hair-like villar protrusions parallel to cyst wall.

**Key words:** Microscopical - serological -ultrastructure - *Sarcocystis species* - sheep – Assiut.

### INTRODUCTION

*Sarcocystis* is one of the most prevalent obligate intracellular protozoan parasites belong to Apicomplexan phylum infecting food livestock such as cattle, sheep and goat (Kia *et al.*, 2011). *Sarcocystis spp.* require two hosts to complete its life cycle; with the sexual cycle in the intestine of a carnivore as a definitive host and asexual cycle in the tissues of an herbivore as an intermediate hosts (Dubey, 1976 and Fayer, 2004). After ingestion of oocysts by the intermediate host, *Sarcocystis spp.* undergoes asexual generations in the vascular endothelial cells in all parts of the body with eventual formation of mature sarcocysts in the host's muscles. The definitive host is infected due to ingestion of the *Sarcocystis* infected muscular tissue, which results in the intestinal generation of oocysts (Fayer *et al.*, 2015).

*Sarcocystis* are common parasites of a broad range of vertebrates. 189 different species can infect

mammals, birds, reptiles, and fish even human (Odening, 1998). Infections are recognized to occur in all parts of the world and farm animals are intermediate hosts for a number of species (Dubey *et al.*, 1989a and Hettiarachchi and Rajapakse, 2008). *Sarcocystis species* infect skeletal muscle, cardiac muscle, and smooth muscle and are rarely found in other tissues (Fayer, 2004).

Sheep are the intermediate host for four species: *S. tenella* (*S. ovis*) and *S. arieticanis* that are known as pathogenic parasites, which form muscular microcysts transferred by dogs. *S. gigantea* (*S. ovifelis*) and *S. medusiformis* are non-pathogenic and form macrocysts transferred by cats (Tenter, 1995; Heckerroth and Tenter, 1998 and Dubey *et al.*, 2015). Sheep become infected with the parasite via ingesting sporocysts or sometimes sporulated oocysts existed in the food or water (Dubey and Lindsay, 2006). Natural infections by *Sarcocystis* in domestic sheep have been investigated in various countries throughout the world, with prevalence ranging from 9 to 100% depending on the detection methodology (Dubey *et al.*, 2015).

The severity of clinical signs in domesticated animals depends on the species of parasite and number of

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digested oocysts. *S. tenella* is the most pathogenic *Sarcocystis* species in sheep. It can cause anorexia, fever, decreased weight gain, anemia and has been associated with abortions in ewes. Neurologic signs including encephalomyelitis, muscle weakness, hind limb paresis and ataxia have been seen in naturally infected sheep. After recovery from the acute illness, some sheep may lose their wool. Sudden deaths can also occur without other symptoms. Experimentally, *S. arieticanis* is also pathogenic but somewhat less so than *S. tenella*. In the other hand *S. medusiformis* and *S. gigantea* are nonpathogenic or cause only mild disease (Charleston, 1994 and OIE, 2005).

*S. tenella* causes significant losses in the life stock industry due to death of the animal or abortion of pregnant ewes during acute sarcocystosis, and reduced weight gain, milk and wool production during chronic sarcocystosis (Kia *et al.*, 2011 and Dubey *et al.*, 2015). *S. gigantea* and *S. medusiformis* develop macroscopic cysts in striated muscles of sheep which are considered as causes of economic losses in the sheep industry. The heavily infected sheep meat by macroscopic cysts may be condemned as unfit for human consumption during meat inspection in abattoirs. Economic losses in Spain due to *Sarcocystis* infection to the Spanish sheep industry have been estimated 20 million Euros annually and an average 4 million sheep culled every year (Martínez-Navalon *et al.*, 2012 and Farhang-Pajuh *et al.*, 2014)

Identification of different species of *Sarcocystis* is based on the structure study of the cyst wall by using light or transmission electron microscope of tissue samples from infected hosts, primarily from the skeletal muscle, tongue, heart, diaphragm, and esophagus (Jehle *et al.*, 2009 and Latif *et al.*, 2015).

Several Serological tests such as enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT), complement fixation tests and an agar gel diffusion test has also been used to detect sarcocystosis in animals and humans (Hay and Westwood, 2002; Kalita *et al.*, 2015 and Latif *et al.*, 2015).

The aim of the present study was to determine the microscopical and serological prevalence of natural infection with *Sarcocystis* in the muscles of different organs (tongue, oesophagus, heart, diaphragm and skeletal muscles) of sheep slaughtered in Assiut Gov., Egypt, with identification of the parasite species by light and electron microscope. In addition studying the influence of age, gender of sheep on the infection rate.

## MATERIALS AND METHODS

**Collection of samples:** In this study, Blood and muscle samples were collected from 100 sheep (42

male and 58 female) with age ranged from 6 months to 5 years slaughtered at abattoirs from different locations in Assiut Governorate. Fresh tissue samples of tongue, oesophagus, heart, diaphragm and neck skeletal muscle were collected from each animal in clean separate labeled plastic bags. Samples were labeled separately, kept in icebox and rapidly transported to the lab where refrigerated at 4°C for further investigation. Moreover, blood sample were collected from each animal during slaughtering in clean, labeled centrifuge tube and their sera were separated by centrifugation and stored at -20°C until tested.

**Macroscopic examination:** Each fresh sample was thoroughly examined by naked eye for the presence of macroscopic *Sarcocystis sp.* cysts (Mahran, 2009).

**Microscopic examination:** According to (Mahran, 2009 and Latif *et al.*, 2015), using Squash method "the compress method" where small pieces of fresh muscle measuring 2-3 mm<sup>3</sup> were cut from each tissue sample and squeezed firmly between two glass slides and examined microscopically at 10 x magnifications.

**Transmission electron microscopy (TEM):** Specimens of muscles were prefixed in 2.5% glutaraldehyde solution, diced into 1 mm<sup>3</sup>, then rinsed three times for 15 min each with 0.1 M phosphate buffer (pH 7.4). Post-fixation samples were placed in a cold 1% aqueous osmium tetroxide for 2 h. After rinsing with phosphate buffer again, the specimens were dehydrated in a graded ethanol series of 50–100% and then embedded in Epon 812. Semithin sections were sliced with glass knives on ultramicrotome for orientation and selection of representative tissue specimens and stained with 0.5% Toulidine blue according to Bancroft and Stevens (1993). Ultrathin sections of (500-800 Å) were done using ultramicrotome then contrasted in uranyl acetate and lead citrate, and examined under a (Jeol, CX11) electron microscope in Electron Microscope unit, Assiut University.

### Serological examination:

**Preparation of antigen** According to Hettiarachchi and Rajapakse (2008): antigen prepared from macroscopically visible *Sarcocystis fusiformis* cysts were obtained from the oesophagus of heavily infected buffaloes carcasses at Assiut city Abattoir. They were teased out of the muscle with fine forceps, collected into phosphate buffered saline (PBS) PH 7 and stored at -20 °C. Twenty sarcocysts were collected into tube and washed with saline to remove attached bovine tissue. Using fine forceps the cysts were macerated in 1 mL of PBS at room temperature until a cream coloured suspension formed. A drop of the suspension was examined under the light microscope (x40) to confirm presence of banana-shaped cystozoites. These cystozoites were

centrifuged at 3000 r.p.m for 10 min, resuspended in PBS (PH 7), recentrifuged at 3000 r.p.m for 10 min and the supernatant discarded. The pellet of cystozoites was mixed with about 10 mL of 50% isotonic Percoll solution (Percoll® P1644-25ML, Sigma-Aldrich Co, St. Louis, MO 63103 USA) in a conical tube and centrifuged at 2500 rpm for 10 min. Extraction buffer consisting of 200µL 1% Triton diluted by PBS (PH 7) (TritonX-100, Sigma-Aldrich Co, St. Louis, MO 63103 USA) in PBS was then added to the eppendorf. The sample was mixed well by a whirly mixer for 5 min, while alternatively placing it in ice to avoid heating. The sample was then centrifuged for 10 min at 14000 rpm in 4°C. The resulting supernatant (antigen) was collected into eppendorfs then estimated of protein quantity in the antigen using BioTek® Instruments, Inc. (Center of Medical research in Assiut University) and stored at –20°C.

**Agar gel diffusion test:** According to Hay and Westwood (2002) and Al-Taee *et al.* (2009), Fifteen mL of 1% agarose solution (Bioshop® Canada Inc., Burlington, ON. L7L 6A4) in 8% saline was poured into a Petri dish 9 cm in diameter. Several wells were prepared in the agarose gel at a distance of 3 mm between the wells. Undiluted and untreated serum was used in this study. Each well in the gel was filled with the antigen or serum to be tested. The dish was kept in a moist chamber at room temperature for 4 days and the reaction was observed daily. When a distinct precipitin line was found in the gel, the serum was considered to have the antibody against *Sarcocystis*.

**Statistical analysis:** Statistical analysis was performed by SPSS (ver. 16, IBM, New York, USA) using  $\chi^2$  test with 95% confidence interval, with considering P value (P <0.05) as significant difference (Mirzaei and Rezaei , 2016).

## RESULTS

One hundred sheep muscle samples were examined for *Sarcocystis spp.*, no macroscopic cysts were observed in the tissues sample of sheep by

macroscopic examination. The infection rate of *Sarcocystis spp.* was 93% (93/100) by microscopic examination (Figure 1) and 28% (28/100) by Agar gel diffusion test (Table 1, Figure 2). The infection rate of *Sarcocystis* had very high significant differences between microscopic examination and Agar gel diffusion test. The infection rate of *Sarcocystis spp.* in different muscular tissues of sheep (Table 2), showed very high statistical significant effect on the infection rate ( $\chi^2=19.212$ ,  $p>0.001$ ). The highest infection rate was recorded in the oesophagus (71%) followed by the diaphragm (65%), tongue (58%) and skeletal muscles (53%); the lower rate was recorded in the heart muscles (43%). Concerning the sex, the infection rate of *Sarcocystis spp.* in male sheep was 95.2% (40/42), while in female was 91.4% (53/58) by microscopic examination (Table 3). Infection with *Sarcocystis* associated with age is shown in table 4, higher infection rate among sheep 6 months - 2 years 95% (38/40) than that of sheep between 2-4 years old 92.7% (38/41) and those equal or older than 4 years 89.5% (17/19). No significant differences between infection and age groups or sex of animals were observed.

The ultrastructural evaluation of *Sarcocystis spp.* in sheep was mainly targeted to the cyst walls. The use of TEM allowed the identification of *S. tenella* and *S. arieticanis* in sheep. Examination of *S. tenella* cyst in semithin section showed cyst wall with villar protrusions. Metrocytes were adhered the inner surface of the cyst wall followed by bradyzoites in groups separated from each other's with fine septa (Figure 3). Examination of *S. tenella* cyst with TEM showed that the cyst wall consisted of palisade-like villar protrusions. The ground substance is located directly under the primary cyst wall. Bradyzoites found in inner side of ground substance. Host mitochondria and host cells found near cyst surface (Figure 3, 4). Examination of *S. arieticanis* cyst with TEM showed that the cyst wall consisted of hair-like villar protrusions parallel to cyst wall. The ground substance is located directly under the primary cyst wall. Sections of bradyzoites found in inner side of ground substance contain ampylopectin granules. Host mitochondria found near cyst surface (Figure 5).

**Table 1:** Comparative efficiency of techniques used for detection of *Sarcocystis spp.* in sheep.

Technique	No. of examined sheep	No. of positive***	%	$\chi^2$	P
Light microscopy	100	93	93	88.4	<0.0001
Agar gel diffusion test	100	28	28		

\*\*\* Very high significant differences (P<0.0001)

**Table 2:** Infection rate of *Sarcocystis spp.* in different organs of sheep using light microscope.

Organ	No. of examined	No. of positive***	%	$\chi^2$	P
Oesphagus	100	71	71		
Tongue	100	58	58		
Heart	100	43	43	19.212	<0.001
Diaphragum	100	65	65		
Skeletal muscle	100	53	53		

\*\*\* Very high significant differences (P<0.001)

**Table 3:** Effect of sex on the infection rate of *Sarcocystis spp.* in sheep using light microscope.

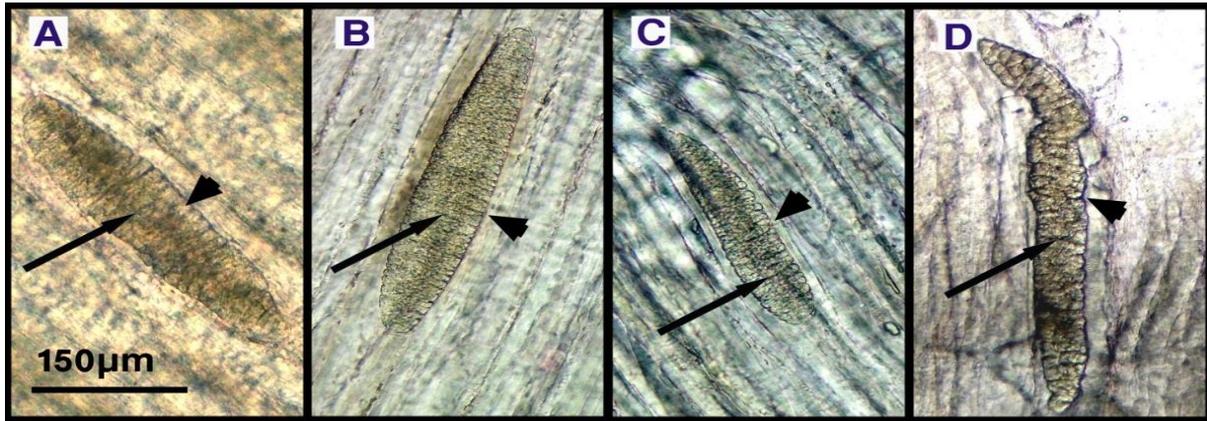
Sex of sheep	No. of examined sheep	No. of positive	%	$\chi^2$	P
Female	58	53	91.4		
Male	42	40	95.2	0.557	>0.05
Total	100	93	93		

Insignificant differences (P>0.05)

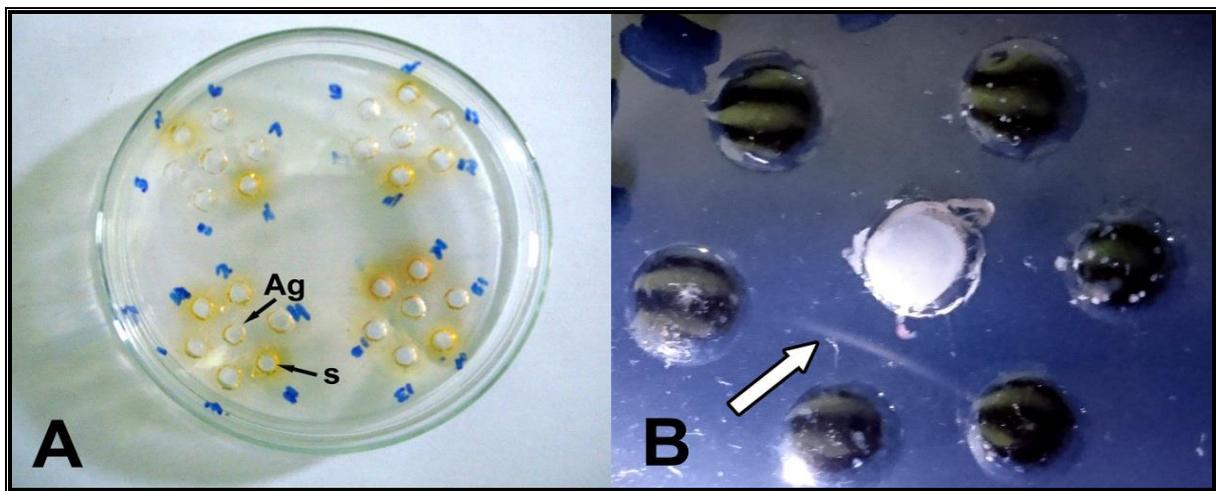
**Table 4:** Effect of age on the infection rate of *Sarcocystis spp.* in sheep using light microscope.

Age of sheep	No. of examined sheep	No. of positive	%	$\chi^2$	P
6 m - 2 y	40	38	95		
2 to <4 year	41	38	92.7		
≥4 year	19	17	89.5	0.615	>0.05
Total	100	93	93		

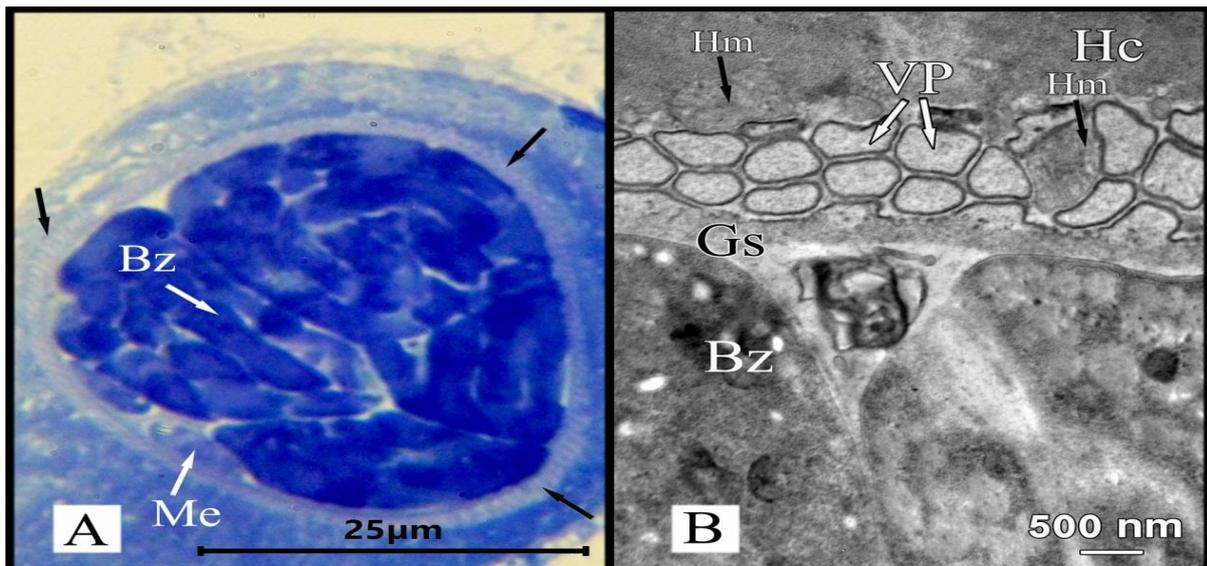
Insignificant differences (P>0.05)



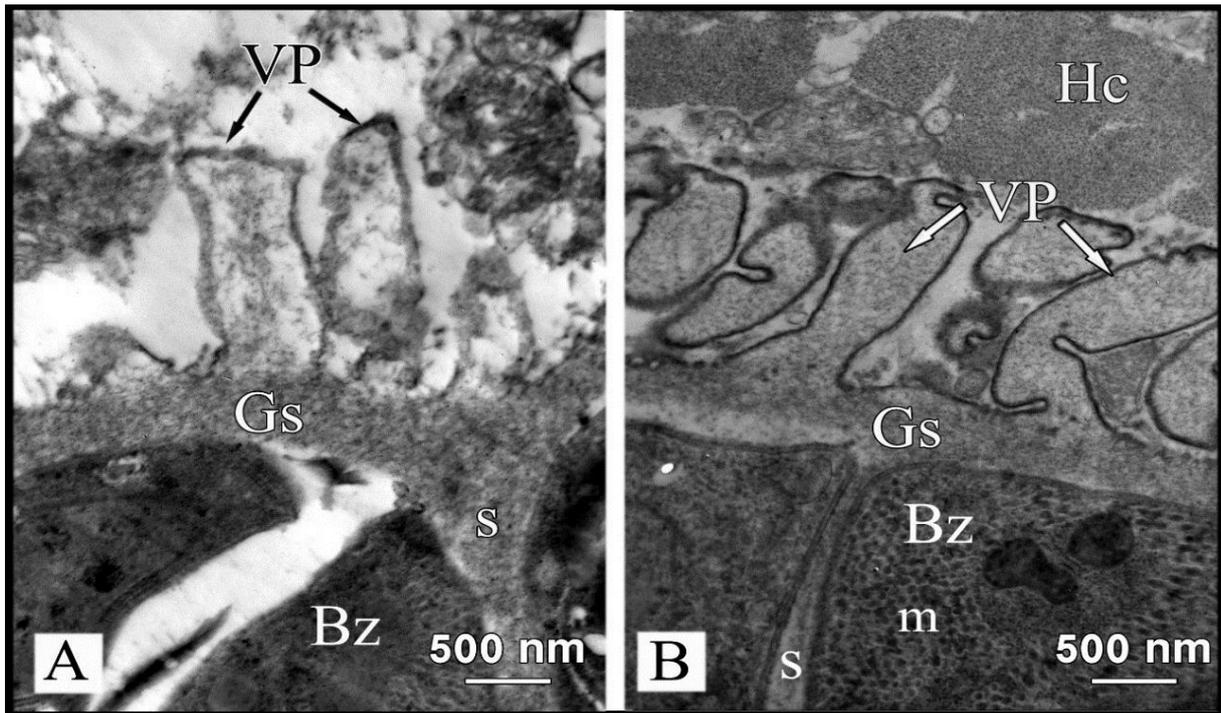
**Figure 1:** Fresh *Sarcocystis* spp. cysts in the muscles of sheep compressed between two slides, showing cyst parallel to muscle fibers; Note spindle shaped cyst (arrow head) and septa (arrow) dividing the internal compartments are seen as dark structures (X 10).



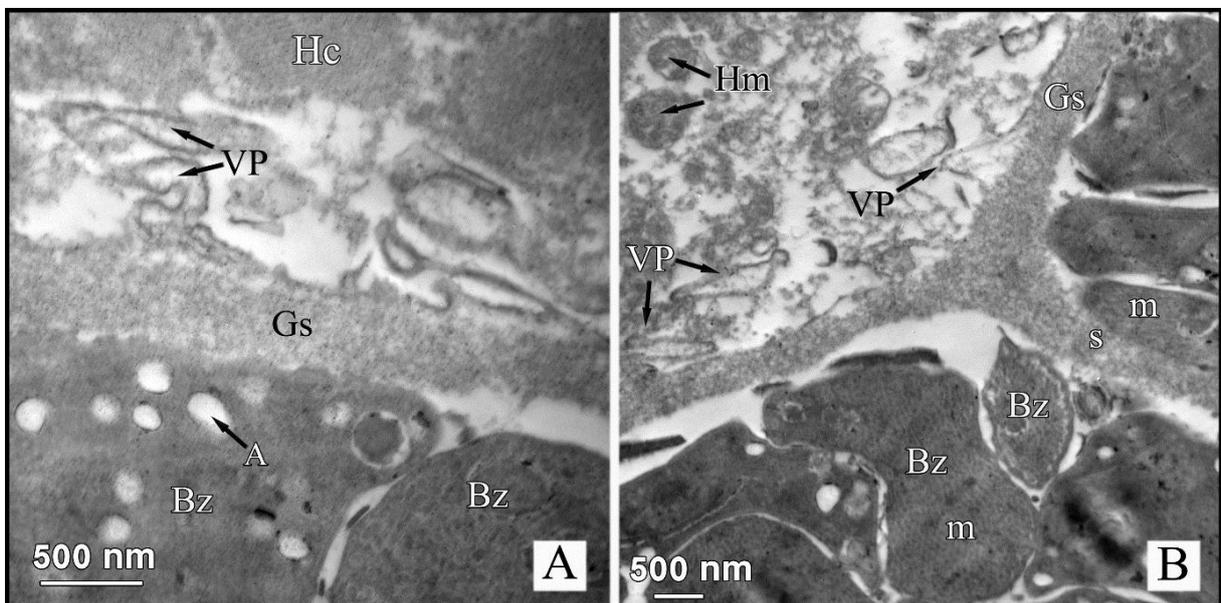
**Figure 2:** Agar gel diffusion test for diagnosis of *Sarcocystis* spp. in sheep. **A.** Agarose gel with *Sarcocystis* antigen (Ag) in central well and sheep tested sera (S) in peripheral wells in petri dish. **B.** showing precipitin line (arrow) between positive serum sample and antigen.



**Figure 3:** Morphological characteristics of *Sarcocystis tenella* cysts from sheep muscle. **(A)** Semithin section of *Sarcocystis tenella* in muscle of sheep, showing cross section of cyst inside muscle fiber showing villar protrusions (arrows). Metrocyst (Me) found in the inner side of wall followed by bradyzoites (Bz) in chambers. Toluidine blue stain (X100). **(B)** Transmission electron micrograph (TEM) showing cross-section of a sarcocyst with cross sections of villar protrusions (VP), ground substance (Gs) and bradyzoites (Bz) in inner side of ground substance. Note host mitochondria (Hm) and host cells (Hc) near cyst surface (14000X).



**Figure 4:** TEM of *Sarcocystis tenella* from muscle of sheep. (A) TEM showing longitudinal sections showing palisade-like villar protrusions (VP). Sections of bradyzoites (Bz) found inner side of ground substance (Gs) separated by septa (S) (19000X). (B) TEM showing Palisade-like villar protrusions (VP). Sections of bradyzoites (Bz) found in inner side of ground substance (Gs) contain micronemes (m) separated by septa (S) Note host cell (Hc) near cyst surface (19000X).



**Figure 5:** TEM of *Sarcocystis arieticanis* sarcocysts from sheep muscle. (A) Showing hair-like villar protrusions (VP) parallel to cyst wall. Sections of bradyzoites (Bz) found in inner side of ground substance (Gs) contain several ampylopectin granules (A). Note host cell (Hc) near cyst surface (29000X). (B) Showing sections in villar protrusions (VP). Different sections of bradyzoites (Bz) found inside cyst separated by septa (s). Bradyzoites in inner side of ground substance (Gs) contain micronemes (m). Note host mitochondria (Hm) near cyst surface (14000X).

## DISCUSSION

*Sarcocystis spp.* are the most common parasites in domestic ruminants and some of them can generate significant economic losses due to decreased production rates or death of the most severely infected animals (Martínez-Navalón *et al.*, 2012 and Hu *et al.*, 2017).

No macroscopic cysts were observed in the tissues sample of the 100 sheep by macroscopic examination in our study. This result is in line with those recorded before in Dakahlia Governorate, Egypt (Abd El-Kader, 2008); Iran (Hajimohammadi *et al.*, 2014); Brazil (Bittencourt *et al.*, 2016) and Iraq (Zangana and Hussein, 2017), whereas Mahran (2009) and El-Mishmishy (2017) reported an infection rate of 9.9% and 0.74% in sheep in Red Sea and Cairo Governorates, respectively. Moreover, Martínez-Navalón *et al.* (2012) reported an infection rate of 12% in Spain and (Bahari *et al.*, 2014) 5% in Iran. The absence of macroscopic sarcocysts in the present study, in comparison with high infection rate of microscopic sarcocysts may be due to the fact that such cysts are of feline origin which characterized by production of low number of sporocysts. The results were in agreement with, Dubey *et al.* (1989b) who mentioned that *Sarcocystis species* transmitted by felids have been found less frequent than those transmitted by canids because of low oocyst production in those hosts. Also, Fouly (1997) reported that no sporocysts of *Sarcocystis* are found in all collected faecal samples of cats in Assiut.

In the present study, the infection rate of *Sarcocystis* in sheep was 93% (93/100) by microscopic examination. This result agreed with those recorded by El-Mishmishy (2017) who mentioned that the prevalence of *Sarcocystis spp.* cysts at 3 Egyptian Provinces (Dakahlia, Damietta and Cairo) was 95.37%. Moreover studies carried out from different countries have reported similar prevalence of infection in sheep, in Ethiopia (Woldemeskel and Gebreab, 1996) reported an infection rate of 93.6%, also (Bittencourt *et al.*, 2016) and (Hu *et al.*, 2017) reported an infection rates of 95.8% and 91.9% in sheep in Brazil and china, respectively. The high infection rate of infection by *Sarcocystis* in the present work might be due to presence of the examined sheep in rural areas in close association with dogs which contaminate the animal's food with large number of *Sarcocystis* sporocysts. In addition to that the sporocysts are mostly infective already when passed in the faeces. The result were in agreement with Nourollahi Fard *et al.* (2009) and Latif *et al.* (1999) who recorded that carnivorous such as canine, infect environment via faeces by passing 200 million oocyst of *Sarcocystis* during infection period.

Whereas our result was lower than those previously reported by Whaeab and Faraj (2016) 100%, Zangana

and Hussein (2017) 97.5% in Iraq and Hajimohammadi *et al.* (2014) 97.14%, Mirzaei and Rezaei (2016) 100% in Iran. On the contrary, lower values were recorded by various authors (61.41%) in Assiut Governorate (Taher, 1985); 81.8% at Shalatin abattoir Red Sea Governorate (Mahran, 2009); 65.15% in New-Valley Governorate (Elbadre *et al.*, 2014) and from different countries 47.32% in Turkey (Ozkayhan *et al.*, 2007), 81.5-90% in K.S.A. (Al Quraishy *et al.*, 2014), 86% in Malaysia (Latif *et al.*, 2015) and 72.2% in India (Gopal *et al.*, 2016). The variation in these results may be attributed to the difference in the diagnostic technique used, study locality, management conditions and number of samples (Mahran, 2009; Farhang-Pajuh *et al.*, 2014; Latif *et al.*, 2015 and Gopal *et al.*, 2016).

The infection rate of *Sarcocystis spp.* in sheep was 28% (28/100) by Agar gel diffusion test. So, using antigen prepared from macroscopically visible *Sarcocystis fusiformis* cysts could be used in serological diagnosis of sheep *Sarcocystis*. This finding agrees with that observed by Dubey and Fayer (1983) who recorded that antigen prepared from *Sarcocystis* of one host generally cross reaction with antibodies against homologous and heterologous species of *Sarcocystis*. Similar results reported by Al-Taei *et al.* (2009) who revealed that the rate of *Sarcocystis sp.* infection in 100 sheep was 82% by Modified Enzyme linked Immunosorbent Assay (ELISA) and 25% by Agar gel precipitation test in Iraq. Significant differences were detected in sensitivity of these serological tests.

Our results revealed that the infection rate of *Sarcocystis* in sheep was 93% by microscopical examination while was 28% by Agar gel diffusion test. These differences may be due to variation in the intensity of infection in these animals which in turn effect of the amount of the released antibodies that can be detected by Agar gel diffusion test. This finding agree with Gasbarre *et al.* (1984) who detected that IgG antibody levels of sheep inoculated with *Sarcocystis ovicanis* was slightly delayed increase (6 to 8 weeks after inoculation was done). Also, measurable IgM antibody response was not seen in sheep.

Concerning the infection rate of *Sarcocystis spp.* in different muscular tissues of sheep, this study showed variable rates of distribution of sarcocysts in the tissues with very high statistical significant effect on the infection rate ( $\chi^2=19.212$ ,  $p>0.001$ ). The highest infection rate was recorded in the oesophagus (71%) followed by the diaphragm (65%), tongue (58%) and skeletal muscles (53%); the lower rate was recorded in the heart muscles (43%). In Red Sea Governorate, Mahran (2009) made similar observation he revealed that maximum sarcocysts were found in the muscles of oesophagus in sheep (71.1%) followed the

diaphragm, tongue, skeletal muscles and the heart muscles (54.9%, 48.6%, 40.7% and 36.9%), respectively. Likewise, Hu *et al.* (2017) in China mentioned that the distribution of the sarcocysts was 84.9% in esophagus, 32.6% in tongue, 57% in diaphragm, 20.9% in heart and 79.1% in skeletal muscles. These differences in the infection rate of the organs of sheep might be due to various external factors, such as *Sarcocystis* spp. responsible for infection, or differences in the study locality and nutritional status of the hosts that may lead to variations in the immunity of the host against infection (Latif *et al.*, 2015).

Observation on the variation due to the sex of sheep on infection rate of sarcocysts infection, showed that males were more infected 95.2% than females 91.4%, but there was no significant difference between different sexes. This result correlated with the observation of Farhang-Pajuh *et al.* (2014) that found that the prevalence was 55.17% and 15.20% among male and female sheep, respectively. Likewise, Hajimohammadi *et al.* (2014); Mirzaei and Rezaei (2016) and Zangana and Hussein (2017) found no significant difference between males and females. However, in a study by El-Mishmishy, (2017) he reported that ewes had a higher prevalence of infection (99%) than males (94.55%). These differences may be due to difference in the management conditions in such localities.

In relation to age; the infection rate of *Sarcocystis* spp. in sheep showed higher infection rate among sheep 6 months- 2 years (95%) than that of sheep between 2-4 years old (92.7%) and those equal or older than 4 year (89.5%), no significant difference was observed. This result agreed with Hajimohammadi *et al.* (2014) and Mirzaei and Rezaei (2016) that found no significant difference in infection of younger and older animals. While disagreed with Mahran (2009); Elbadre *et al.* (2014) and El-Mishmishy (2017) that reported higher infection rate among older sheep than that younger one. These differences may be due to difference in location, number of samples and management conditions.

Although light microscopic examination is commonly used for identification of sarcocysts, electron microscopy has been used to identify *Sarcocystis* spp. according to their wall structure (Hazirolu *et al.*, 2002 and Heckerroth and Tenter, 2007).

In the present study, the use of TEM of *Sarcocystis* wall showed palisade-like villar protrusions which allowed the identification of *Sarcocystis tenella*. While, the cyst wall of *Sarcocystis* that showed hair-like villar protrusions parallel to cyst wall were identified as *Sarcocystis arieticanis*. These results were in agreement with Dubey *et al.* (1989b) and Heckerroth and Tenter (2007) who described *S.*

*arieticanis* with hair-like projections in the walls of their cysts. Similar results reported by Bittencourt *et al.* (2016) and Hu *et al.* (2017) who identified the ultrastructural of *Sarcocystis tenella* and *S. arieticanis* in tissues from sheep in Brazil and China, respectively.

Our results revealed that *S. arieticanis* cysts were septated, similarly as observed by Heydorn and Mehlhorn (1987) and Bittencourt *et al.* (2016) who noted that the septa inside some cysts are sometimes not easily visualized, especially in large cysts with large compartments. In contrary, Dubey *et al.* (1988) described *S. arieticanis* cysts lack of septa in from sheep in the USA which probably due to the low number of analyzed cysts.

In the present work, TEM revealed the mitochondria were seen to be concentrated near the villar protrusions of *Sarcocystis* in some host cells of muscle. This finding was similar to that mentioned by Abdel Mawla (1990) who suggested the presence of mitochondria with *Sarcocystis* infection due to disappearance of myofibrillar pattern in the precystic zone, then loss of the typical linear distribution of the muscle mitochondria and causes their crowding in the ultrastructure study.

Our results revealed a high infection rate of *Sarcocystis* in sheep. Therefore, we recommend that feed supplies of sheep must be protected from faecal contamination by carnivores. Also, it has of great importance the farmers to be trained not to feed their dogs and cats with uncooked meat in order to be effectively broken of infection cycle between sheep and carnivores.

## REFERENCES

- Abd El-Kader, A.I.E. (2008): Studies on tissue parasites of farm animals in Dakahlia Province. Thesis (M.S.) Faculty of Veterinary Medicine, Department of Parasitology- Mansoura University.
- Abdel Mawla, M.M. (1990): Ultrastructure of the cyst wall of *Sarcocystis lindemanni* with pathological correlations. J. Egypt. Soc. Parasitol., 20 (1): 319 – 325.
- Al Quraishy, S.; Morsy, K.; Bashtar, A.R.; Ghaffar, F.A. and Mehlhorn, H. (2014): *Sarcocystis arieticanis* (Apicomplexa: Sarcocystidae) infecting the heart muscles of the domestic sheep, *Ovis aries* (Artiodactyla: Bovidae), from KSA on the basis of light and electron microscopic data. *Parasitology research*, 113(10): 3823-3831.
- Al-Taei, A.F.; Al-Hyali, N.S. and Al-Badree, M.S. (2009): Seroprevalence of antibodies against *Sarcocystis gigantea* in different hosts in Ninevah governorate. Iraqi Journal of Veterinary Sciences 23 (1): 107-112.

- Bahari, P.; Salehi, M.; Seyedabadi, M. and Mohammadi, A. (2014): Molecular identification of macroscopic and microscopic cysts of *Sarcocystis* in sheep in North Khorasan province, Iran. International journal of molecular and cellular medicine, 3(1): 51-56.
- Bancroft, J.D. and Stevens, A. (1993): Theory and practice of histological techniques. 3<sup>rd</sup> ed. Churchill Livingstone. Edinburgh, London, Melbourne and New York.
- Bittencourt, M.V.; Meneses, I.D.; Ribeiro-Andrade, M.; de Jesus, R.F.; de Araujo, F.R. and Gondim, L.F. (2016): *Sarcocystis* spp. in sheep and goats: frequency of infection and species identification by morphological, ultrastructural, and molecular tests in Bahia, Brazil. Parasitol Res.; 115(4):1683-1689.
- Charleston, W.A.G. (1994): *Toxoplasma* and other protozoan infections of economic importance in New Zealand. New Zealand journal of zoology, 21(1): 67-81.
- Dubey, J.T. (1976): A review of *Sarcocystis* of domestic animals and of other coccidia of cats and dogs. Journal of the American Veterinary Medical Association, 169(10): 1061-1078.
- Dubey, J.P. and Fayer, R. (1983): *Sarcocystis*. British Vet. J., 139; 371-377.
- Dubey, J.P. and Lindsay, D.S. (2006): Neosporosis, toxoplasmosis, and sarcocystosis in ruminants. Vet Clin. Food Anim., 22:645-671.
- Dubey, J.P.; Lindsay, D.S.; Speer, C.A.; Fayer, R. and Livingston, C.W.Jr. (1988): *Sarcocystis arieticanis* and other *Sarcocystis* species in sheep in the United States. J Parasitol 74:1033-1038
- Dubey, J.P.; Speer, C.A. and Charleston, W.A.G. (1989a): Ultrastructural differentiation between *Sarcocystis* of *Sarcocystis hirsuta* and *S. hominis*. Vet Parasitol (34):153-157.
- Dubey, J.P.; Speer, C.A. and Fayer, R. (1989b): Sarcocystosis of animals and man. CRC Press Inc, Boca Raton, Florida. 105-145.
- Dubey, J.P.; Calero-Bernal, R.; Rosenthal, B.M.; Speer, C.A. and Fayer, R. (2015): Sarcocystosis of animals and humans. 2<sup>nd</sup> edition. CRC Press, Boca Raton, Florida. p 1-481.
- Elbadre, M.A.; Osman, F.A. and Hussien, S.M. (2014): Studies on *Sarcocystis* in Sheep and Goats using Serological Survey in New Valley Governorate Egypt. IJAVMS, 8(Issue 5): 150-153.
- El-Mishmishy, B.M.M. (2017): Molecular characterization of *Sarcocystis* species in sheep. Thesis (Ph. D.) Faculty of Veterinary Medicine. Department of parasitology-Mansoura University.
- Farhang-Pajuh, F.; Yakhchali, M. and Mardani, K. (2014): Molecular determination of abundance of infection with *Sarcocystis* species in slaughtered sheep of Urmia, Iran. Veterinary Research Forum, 5 (3):181-186.
- Fayer, R. (2004): *Sarcocystis* spp. in human infections. Clin Microbiol Rev 17:894-902.
- Fayer, R.; Esposito, D.H. and Dubey, J.P. (2015): Human infections with *Sarcocystis* species. Clin Microbiol Rev 28:295-311.
- Fouly, E.A. (1997): Studies on parasites of cats in Assiut Governorate. Ph. D. Thesis, Fac. of Vet. Med., Assiut University.
- Gasbarre, L.C.; Suter, P. and Fayer, R. (1984): Humoral and cellular immune responses in cattle and sheep inoculated with *Sarcocystis*. American Journal of Veterinary Research 45(8):1592-1596.
- Gopal, K.; Pazhanivel, N.; Kumar, V. and Thangathurai, R. (2016): Some observations on *Sarcocystis* Infection in Sheep. Indian Vet. J., 93 (10): 81-82.
- Hajimohammadi, B.; Zohourtabar, A.; Fattahi Bafghi, A.; Omidpanah, A.; Rayati, T.; Ghasemi, A. and Sazmand, A. (2014): Occurrence and distribution of *Sarcocystis* parasite isolated from sheep in Yazd province, Iran. Journal of Community Health Research, 3(3): 205-210.
- Hay, F.C. and Westwood, O.M. (2002): Practical Immunology. 4<sup>th</sup> ed. Black-Well Scientific Publications, London. P 1-400.
- Haziroglu, R.; Guvenc, T. and Tunca, R. (2002): Electron microscopical studies on cysts of *Sarcocystis arieticanis* within cardiac muscle of naturally infected sheep. Parasitology Research 89: 23-25.
- Heckerroth, A.R. and Tenter, A.M. (1998): Comparison of immunological and molecular methods for the diagnosis of infections with pathogenic *Sarcocystis* species in sheep. Tokai J Exp Clin Med.; 23: 293-302.
- Heckerroth, A.J. and Tenter, A.M. (2007): Sarcocystiosis. In: Ortega-Mora LM, Gottstein B, Conraths FJ, Buxton D. (eds.): Protozoal Abortion in Farm Ruminants. CAB International, UK. 172-232.
- Hettiarachchi, D.C. and Rajapakse, R.P.V.J. (2008): Antigenic analysis of bovine *Sarcocystis* spp. in Sri Lanka. Journal of the National Science Foundation of Sri Lanka 36 (3):239-244.
- Heydorn, A.O. and Mehlhorn, H. (1987): Fine structure of *Sarcocystis arieticanis* Heydorn, 1985 in its intermediate and final hosts (sheep and dog). Zentralbl Bakteriol Mikrobiol Hyg A 264:353-362
- Hu, J-J.; Huang, S.; Wen, T.; Esch, G.W.; Liang, Y. and Li, H-L. (2017): *Sarcocystis* spp. in domestic sheep in Kunming City, China: prevalence, morphology, and molecular characteristics. Parasite, (24) 30:1-8.

- Jehle, C.; Dinkel, A.; Sander, A.; Morent, M.; Romig, T.; Luc, P.V.; De, T.V.; Thai, V.V. and Mackenstedt, U. (2009): Diagnosis of *Sarcocystis* spp. in cattle (*Bos taurus*) and water buffalo (*Bubalus bubalis*) in Northern Vietnam. *Vet Parasitol* 23:314–320.
- Kalita, M.; Sarmah, P.C. and Singh, S.K. (2015): Evaluation of modified agglutination test in detection of antibodies against *Sarcocystis cruzi* and *Sarcocystis hirsuta* of cattle. *International Journal of Recent Scientific Research* 6, Issue, 8, pp.5968-5970.
- Kia, E.B.; Mirhendi, H.; Rezaeian, M.; Zahabiun, F. and Sharbatkhori, M. (2011): First molecular identification of *Sarcocystis miescheriana* (Protozoa, Apicomplexa) from wild boar (*Sus scrofa*) in Iran. *Experimental parasitology*, 127(3): 724-726.
- Latif, B.M.A.; Al-Delemi, J.K.; Mohammed, B.S.; Al-Bayati, S.M. and Amiry, A.M. (1999): Prevalence of *Sarcocystis* spp. in meat production animals in Iraq. *Vet Parasitol*; 84(1-2):85-90.
- Latif, B.; Kannan Kutty, M.; Muslim, A.; Hussaini, J.; Omar, E.; Heo, C.C.; Rossle, N.F.; Abdullah, S.; Kamarudin, M.A. and Zulkarnain, M.A. (2015): Light microscopy and molecular identification of *Sarcocystis* spp. in meat producing animals in Selangor, Malaysia. *Tropical Biomedicine* 32(3): 444–452.
- Mahran, O.M. (2009): *Sarcocystis* infection in sheep and goats slaughtered in Shalatin Abattoir, Red Sea Governorate, Egypt. *Assiut Veterinary Medical Journal*, 55(121): 341-355.
- Martínez-Navalon, B.; Anastasio-Giner, B.; Cano-Fructuoso, M.; Sanchez- Martínez, P.; Liopis-Morant, A. and Perez-Castarlenas, B., et al. (2012): *Sarcocystis* infection: a major cause of carcass condemnation in adult sheep in Spain. *Spanish J Agric Res.*, 10(2):388–392.
- Mirzaei, M. and Rezaei, H. (2016): The role of sheep in the epidemiology of *Sarcocystis* spp. in Tabriz area northwest of Iran. *J Parasit Dis.*, 40(2):285-288.
- Nourollahi Fard, S.R.; Asghari, M. and Nouri, F. (2009): Survey of *Sarcocystis* infection in slaughtered cattle in Kerman, Iran. *Trop Anim Health Prod*; 41(8):1633-1636.
- Odening, K. (1998): The present state of species systematic in *Sarcocystis* Lankester, 1882 (Protista, Sporozoa, Coccidie). *Systematic Parasitology* 41, 209–233.
- OIE /Center of Food Security and Public Health (2005): *Sarcocystosis*. CFSPH, Iowa; 1–6.
- Ozkayhan, M.A.; Karaer, Z.; Ilkme, A.N. and Atmaca, H.T. (2007): The prevalence of *Sarcocystis* species in sheep slaughtered in municipality slaughterhouse in Kirikkale. *Türkiye Parazitol. Derg.* 31 (4): 272–276.
- Taher, G.I.A. (1985): Studies on *Sarcocystis* of animals in Assiut province. Thesis (M.S.) Faculty of veterinary medicine department of parasitology, Assiut University.
- Tenter, A.M. (1995): Current research on *Sarcocystis* species of domestic animals. *Int J Parasitol* 25(11): 1311- 1330.
- Whaeeb, S.T. and Faraj, A.A. (2016): Molecular Identification and phylogeny of Microscopic *Sarcocystis* Sheep in Baghdad Province. *Int. J. Adv. Res. Biol. Sci.* 3(12): 50-56.
- Woldemeskel, M. and Gebreab, F. (1996): Prevalence of *Sarcocysts* in livestock of northwest Ethiopia. *Zentralbl Vet Med [B]* 43(1):55–58.
- Zangana, I.K. and Hussein, S.N. (2017): Prevalence of *Sarcocystis* Species (*Sarcocystis ovis* and *Sarcocystis capricanis*) in Tongue Muscle of Sheep and Goats in Duhok Province, Kurdistan Region, North Iraq. *Aro-The Scientific Journal of Koya University*, 5(1), 36-40.

## دراسات مجهرية ومصلية مع توصيف التركيب الدقيق لأنواع الساركوسيست في الأغنام في أسيوط

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أجريت هذه الدراسة لتحديد مدى انتشار طفيل الساركوسيست في الأغنام في أسيوط. وقد تم فحص عدد ١٠٠ من الأغنام لطفيل الساركوسيست وقد وجد ان معدل الإصابة ٩٣٪ (١٠٠/٩٣) باستخدام الفحص المجهرى و ٢٨٪ (١٠٠/٢٨) باستخدام اختبار الانتشار خلال جل الأجار. أظهرت هذه الدراسة أن هناك فروق معنوية كبيرة جدا لمدى انتشار طفيل الساركوسيست باستخدام الفحص المجهرى واختبار الانتشار خلال جل الأجار. لم يتم ملاحظة أي من الساركوسيست العيني بالفحص الظاهري. وقد وجد تأثير معنوي إحصائي كبير على انتشار طفيل الساركوسيست في مختلف عينات عضلات الأغنام. وقد سجلت أعلى نسبة إصابة في المريء (٧١٪) تليها الحجاب الحاجز (٦٥٪) واللسان (٥٨٪) والعضلات الهيكلية (٥٣٪) وعضلات القلب (٤٣٪). وكانت نسبة الإصابة بطفيل الساركوسيست في الذكور ٩٥,٢٪ (٤٢/٤٠) بينما في الإناث كانت ٩١,٤٪ (٥٨/٥٣) عن طريق الفحص المجهرى. وقد تم الكشف عن ارتفاع معدل الإصابة بطفيل الساركوسيست في الأغنام في عمر أقل من سنتين ٩٥٪ (٤٠/٣٨) عن الأغنام بين عمر ٢-٤ سنوات ٩٢,٧٪ (٤١/٣٨) وأولئك الذين اعمارها تساوي أو أكبر من ٤ سنوات ٨٩,٥٪ (١٩/١٧). لم يلاحظ أي فرق معنوي بين العدوى في الفئات العمرية المختلفة أو جنس الحيوانات. وقد ساعد استخدام المجهر الإلكتروني على تحديد نوع الساركوسيست تينيليا والساركوسيست أريتيكانييس في الأغنام. حيث تبين وجود إمتدادات زغيبية مثل الحواجز بفحص جدار الساركوسيست تينيليا في حين أظهر فحص جدار الساركوسيست أريتيكانييس وجود إمتدادات زغيبية تشبه الشعيرات على سطح الجدار وتكون تقريبا موازية للسطح.