International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 18-07-2022, Accepted: 05-08-2022, Published: 20-08-2022
Volume 7, Issue 8, 2022, Page No. 92-101

Morphological & epidemiological study of sarcocystis parasite in sheep in Al-Diwaniyah Governorate – Iraq

Murtadha Azoz[1], Khaled Alshaebani[2]
Department of Biology, University of Al-Qadisiyah, College of Education, Al-Qadisiyah-Iraq

Abstract
Sarcocystis is a disease of Both animals and humans (Máca & González-Solís, 2022) [30] in which the parasite needs two hosts to complete its life cycle (Prakas *et al*., 2020) [41]. A definitive carnivore host, and an intermediate host, herbivores such as sheep (Pipia *et al*., 2016) [37] The study included the collection of 415 heads of sheep, to verify the presence of parasite Sarcocystis. A macroscopic examination was conducted on the esophagus, tongue, heart and diaphragm of sheep after slaughter to ensure the presence of macroscopic cysts of the parasite, where the percentage of macroscopic infections was recorded at 7.46%. 0.24% for the diaphragm. The samples were examined methods, the microscopic method, which included three methods, namely, digestion with pepsin, known as artificial digestion, The method of a garlic press, By pressing a glass slide, and the methods showed positive results. The method proved the industrial digestion to be the most efficient of its peers, as it recorded 94%, garlic puree recorded 92%, and the method of pressing the slides 87%, and the results of the microscopic examination reached by the current study showed for all samples of 415 heads of sheep that were collected from massacres Al-Qadisiyah Governorate - Iraq. The study also showed that there are significant differences under the level of significance ($P<0.05$). In the macroscopic cysts injuries between males and females. The percentage of male injuries is 2.07%, female injuries it reached 11.71%. While no significant differences were recorded in the microscopic injuries between males and females (94.30 **,**95.94%) respectively. When studying the differences in the rate of injuries depending on the age group, it was also found that there were significant differences between the ages of sheep less than 18 months, and the rate of infection was 3.19%, and between sheep aged above 18 months recorded 16.54%, and no significant differences were recorded between age groups in microscopic injuries. For less than 18 months, the infection rate was 94.32%, and for more than 18 months it was 96.99%.

Keywords: Morphological, sarcocystis parasite, Al-Diwaniyah Governorate

Introduction
Sarcocystis is one of the primary parasites (Franssen *et al*., 2019) [25]. that causes Sarcocystosis (Dubey *et al*., 2016) [16]. The sacs of the parasite are located inside the intermediate host cells (Abdullah, 2021) [2], and it belongs to the phylum Apicomplexan (Franssen *et al*., 2019) [25]. order Eucoccidiorida (Shnawa & Swar, 2021) [42]. family sarcocystidae (Alhayali *et al*., 2020) [8]. The parasite was first recorded in 1843 AD by the Swiss researcher Miescher (Odening, 1998) [36].as it was seen in the smooth muscles of domestic mice (Lakshmi *et al*., 2013) [27].In the form of white stringy bags, without giving them a scientific name (Fayer *et al*., 2015) [23]. It is spindle-shaped or oval (Metwally *et al*., 2019) [32] and is found in the muscles of the body such as the esophagus (Abdel-Ghaffar *et al*., 2009).and skeletal muscle the diaphragm, the heart (Morsy *et al*., 2018) [33].and the neck muscles and the limbs of animals (Metwally *et al*., 2019) [32].and in severe cases it can spread to the muscles of the entire body (Abubakar *et al*., 2013).The effect of parasite toxins on the occurrence of anemia (Buxton, 1998) [12].Fever and loss of appetite (Florin-Christensen & Schnittger, 2018) [24].acute muscle inflammation (Fayer, 2004a) [21].difficulty swallowing food (Florin-Christensen & Schnittger, 2018) [24]. Due to esophagitis and central signs representing paralysis (Bamac *et al*., 2020) [10]. shivering Muscles (Prakas & Butkauskas, 2012) [39].It may cause a miscarriage (Anemia, 2021) [9].and sometimes even death (Lau *et al*., 2014) [28]. Macrocyst are found in two different sizes, the first being the large, Macro cyst (Sudan *et al*., 2019) [43]. which is easily visible to the naked eye (Florin-Christensen & Schnittger, 2018) [24]. The average macroscopic cyst size ranged from 5.0-29.5 x 3.1-7.3 mm (Sudan *et al*., 2019) [43]. Depending on the animal's progression at the age of the injury, the older the injury, the greater the cyst (Prakas *et al*., 2017) [40]. As for the small microscopic cysts, which are not visible to the naked eye, but by the usual microscope (Prakas *et al*., 2021) [38]. The cysts are milky white in color (Al-Saadi *et al*., 2020) [7].They are found in muscle tissue with a length of 5 to 10 mm and a width of 1.30 to 1.90 mm (Farhang-Pajuh *et al*., 2014) [20]. The parasite needs two hosts to complete its life cycle (Sudan *et al*., 2019) [43]. an intermediate host responsible for asexual reproduction includes herbivores such as sheep and cows, pigs, and goats (Fayer, 2004). And A definitive host responsible for the parasite's sexual reproduction (N.S. *et al*., 2011).is undercooked carnivores (Lindsay & Dubey, 2020) [29]. Sheep are intermediate hosts (Swar & Shnawa,

2021) [44]. and are specialized for four types of Sarcocystis, including *S. tenella, S. gigantea, S. arieticanis, S. medusiformis* (Dubey & Rosenthal, 2013) [17].

Aims of study

1. Epidemiological study and prevalence rates of sheep infection with Sarcocystis spp
2. Morphological study of Sarcocystis spp.

Working methods

Practical study

The period specified for the study in question is 8 months, starting from October 1, 2021 and ending on May 31, 2022, then collecting samples from Al-Qadisiyah Governorate, and the study included all the esophagus of sheep.

Collect the sample from the slaughtered animals

Sheep samples were collected from slaughterhouses and butchers in Al-Diwaniyah governorate, and during a comprehensive field study in the whole governorate, 415 samples were collected from slaughtered sheep, with different ages ranging from 6-48 months, based on age diagnosis based on the number of livestock teeth and horns (El-Seify *et al*.2014). Samples were collected at a rate of three days a week.

Sample preservation

After collecting the 415 samples, washing them with water from slaughtering residues, cleaning them well from excreta, the layer of grease around them, blood and others, then placing them using transparent plastic bags and fixing the identification information on them using a soft non-removable pen. The identification information included (gender, sex, age, slaughter area, date) and then transferred to a special freezer for samples, at a freezing point of -40.

Information collection form

An information form for collecting samples was prepared, which included all the details of the animals, including the type, age, gender, date of sample collection, place of collection from the animal, the area of collection, type of injury, is it a microscopic injury, and the sample number.

Laboratory Study

The samples that were collected and saved in advance were transferred to the Graduate Studies Laboratory at the College of Veterinary Medicine - University of Karbala for the purpose of examining the samples.

Examination methods

Samples are examined in two ways

Direct macroscopic examination method

In this way, the sample taken from sheep is examined by the naked eye to look for macroscopic parasite cysts that can be easily seen, and macroscopic injuries are concentrated in the esophagus area of sheep, especially in the elderly, as in Figure (1). After confirming the infection of macroscopic cysts, we take a piece of the affected tissue and keep it in a urea tube filled with formalin, which was previously prepared to save the samples for histological study.

Microscopic examination method

The samples are transported to the laboratory by means of a special refrigerated box to transport the samples for examination. After thawing the samples from the freeze in which they were kept, three small pieces not exceeding 5 g were taken from different areas of the organ to be examined to obtain accurate results and to perform the following three tests:

How is pepsin digested (Artificial Digestion)

For the purpose of using this method, the digestion solution must be prepared by following the method, which was taken from (Collin *et al*., 1980) [13] and includes dissolving 2.6 grams of pepsin with 5 grams of iodine-free NaCl, adding 7 ml of concentrated hydrochloric acid at a concentration of 1.0% The materials were collected in an opaque glass beaker with a capacity of 1000 ml, taking into account the stirring of the solution to enable it to mix well, and then we adjust the acidity of the solution and measure the PH to reach between 1.2 - 1.6. So that the solution is ready for use. For the purpose of using the solution, the method (AL-Masuady, 2009) [5]. was taken 5 g of each sample was taken and cut by scissors into very small pieces for easy digestion with the digester and transferred to a test tube of 15 ml capacity and 10 ml of the solution was added to it. It was transferred to the incubator at a temperature of 40 m for half an hour (Colllins *et al*., 1980). Then we filter the test tube with 3 layers of medical gauze and transfer it to a new test tube of 10 ml capacity to get rid of the related impurities and filter it by centrifuge at a speed of 2000 revolutions/min. For 3 minutes, then withdraw the stuck with a Pasteur pipette and get rid of it, then add 0.5 of the physiological solution to the precipitate Then we take three drops and

place them separately on a glass slide to be examined under a 40X light microscope for the presence of the parasite.

Examination with a garlic press: Garlic Mincer

To use this method, we need a garlic squeezer. We take a small piece of 5 grams from the esophagus or the muscle part to be examined, then cut it into very small pieces using medical scissors or a medical scalpel and put it in the sphincter and squeeze it vigorously so that the liquid substance can come out of the tissues and then take some drops And put it on the glass slide and put the cover on it to be examined under a microscope with a force of 40X and to make sure of the presence or absence of the parasite as mentioned (Al-Saadi *et al*., 2020) [7]

Slide Squash

In the same previous methods, we take a small piece of tissue to be examined, place it directly between two glass slides and press it firmly with the thumb in a simple circular motion, then we raise one of the slides and examine it under a light microscope with a power of 40X. It is considered one of the simple, inexpensive and very fast methods (Elmishmishy, 2017) [19]. In this test**,** The examination technique is proposed by slide crushing method with dyeing the samples with Giemsa dye:

By taking small pieces of tissue (flesh) samples, crushing them with glass slides, and placing them in a Petri dish filled with methyl alcohol for 1 minute. Then transfer the sample to a Petri dish containing Giemsa dye for 5 min. Then it is washed with water and finally, we examine it under a microscope with a magnification of X10 and X40. To clearly show the microscopic cysts

Results

They were collected during the study period, which started from 1-10-2021 until 30-6-20 2022, where 415 heads of sheep were collected in slaughterhouses in Al-Qadisiyah Governorate - Iraq.

The current study showed that there are significant differences between macroscopic and microscopic injuries in sheep under the level of significance (P>0.05). The number of injuries was 395, with a total percentage of injuries of 95.18%, and the percentage of gross injuries amounted to 7.46%, as shown in Table (1).

Table 1: shows the number and percentage of microscopic and macroscopic infections with Sarcocystis in sheep

Type	Type of injury	Number of samples	Number of injuries	%
sheep	microscopic	415	395	95.18 %
	macroscopic	415	31	7.46 %
Statistical analysis	Chi-square: 638.984 degrees of freedom:1 p-value: 0.000001 *There are significant differences under the level of significance (P>0.05).			

Histological sections of sheep's esophagus showed the presence of different sizes of macroscopic bags located between the muscles and surrounded by a layer Muscle fibers and inside each bag many Bradyzoites. And some of these cysts ruptured within the muscle tissue and the spread of Merozoites (Bradyzoites) as in Figure (1).

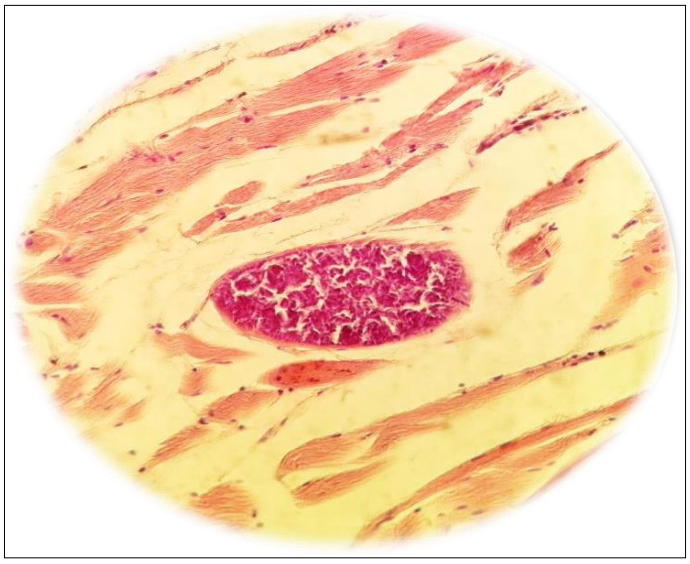

Fig 1: Histological section of the esophageal muscle affected by *S.tenella* showed macroscopic cysts

When comparing the number and percentage of sheep injuries on the basis of the sex variable, the following injury rate was recorded:

Clinical examinations

The results showed by macroscopic examination of the esophagus, tongue, diaphragm and heart of the studied samples for the presence of macroscopic bags of different sizes and shapes, where the macroscopic stages of Sarcocystis parasites are characterized by two sizes, one large and the other small.

The bags appear in different shapes

oval, circular or cylindrical, of white or milky color, with smooth walls, slightly swollen from the middle. Injuries appear more clearly in the esophagus, especially the upper part of the esophagus of sheep, as shown in Figure (2).

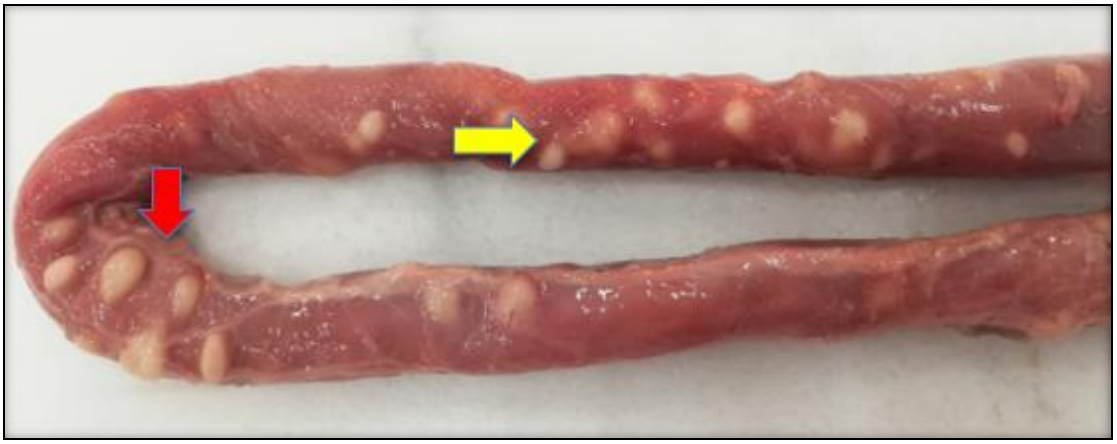

Fig 2: shows the gross infections of the muscular cyst parasite of sheep's esophagus. The red arrow shows the dorsal sacs, while the yellow arrow indicates the submerged sacs under the tissues

The macroscopic bags were studied after they were gently removed from the affected organ for fear of their explosion by the medical scalpel and saved in Petri dishes as in Figure (3) and the dimensions of the macroscopic bags were recorded using a transparent graduated ruler and amounted to 2.4 mm to 6 mm, as shown in Figure (4). One of the macroscopic bags was taken and detonated with a medical scalpel and transferred drops of the contents of the bag onto a glass slide After diluting it with a physiological solution and examining it under a light microscope with a 40× power lens showing the shape of the crescent-shaped sachets and their pointed front. As shown in the figure (5).

Fig 3: Shows the macroscopic bags inside a petri dish of a group of infected sheep

Fig 4: shows the volumes of the macroscopic bags taken from the esophagus of infected sheep using a transparent centimeter ruler

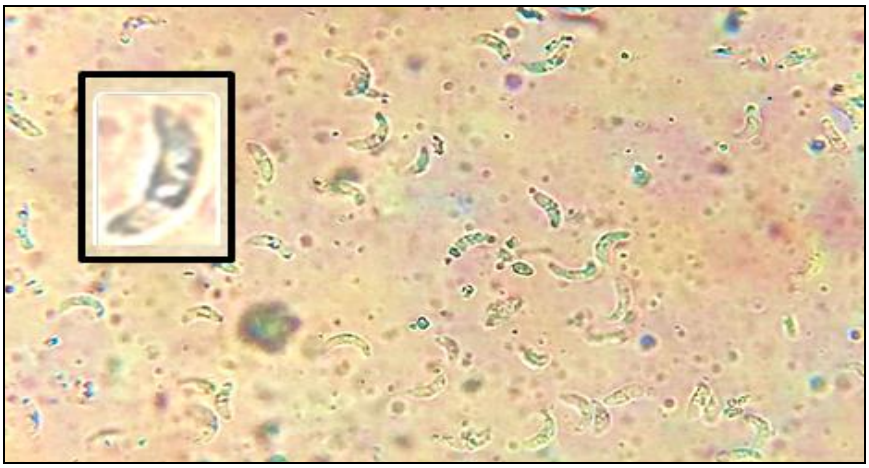

Fig 5: shows the red indicator on one of the slow-breeding instars of Sarcocystis parasite isolated from sheep's esophagus under the X40 light microscope.

Gross diagnostic examinations were divided based on gender, age, and place of the collection as follows :
The relationship of gross injury to the sex of sheep
The results of the current study showed that there are significant differences under the level of significance (P<0.05) between the sex of sheep, and recorded the number of injuries to female sheep, 26 injuries out of 222 samples, at a rate of 11.71%, and recorded the number of injuries to male sheep, 5 injuries out of 193 samples, at a rate of 2.73%, In Table (2). It explains the increase in the incidence of female sheep injuries over males due to the condition that females go through from pregnancy, lactation, and hormonal changes.

Table 2: shows the numbers and percentages of gross infection in Sarcocystis, divided according to the sex of sheep

Sex		Number of samples	Gross Injuries	%
Sheep	male	193	5	2.73 %
	Female	222	26	11.71 %
Sum		415	31	7.46 %
Statistical analysis		Chi-square: 12.427 Degrees of freedom: 2 p-value:0.00200222 * There are significant differences under the significance level (P<0.05)		

Relationship of the specific injury to age
The result of the current study confirmed the existence of significant differences under the significance level of P<0.05 between the ages of the sheep in macroscopic bags infected and clearly, where recorded Chi-square = 23.303 where the sheep were divided according to age into two groups: the first group of sheep at the age of 18 months and more included 22 in macroscopic injuries out of 133 heads of sheep. At a rate of 16.54%, while the percentage of gross injury to sheep under 18 months of age was 9 out of 282 sheep, and at a rate of 3.19% as shown in the table (3).

Table 3: shows the percentage and numbers of gross infections of Sarcocystosis in sheep, divided by age

Type	Age	Number of samples	Gross Injuries	%
Sheep	under 18 months	282	9	3.19 %
	Over 18 months old	133	22	16.54 %
	total summation	415	31	7.46 %
Statistical analysis	Chi-square: 23.303 Degrees of freedom2: p-value: 0.00000871 * There are significant differences under the significance level (P<0.05)			

Assessment of age-sex relationships in macroscopic injury

It was noted from the results of this current study that there were significant statistically significant differences in infection rates between sex and age with a significant significance of P<0.05. The infection rate for females less than 18 months was 5.14%, while the infection rate for females older than 18 months was 22.09%, while the infection rate for males under 18 months was 0.68%, and for males over 18 months the percentage was. 8.51%. As shown in Table No. (4).

Table 4: shows the extent of the discrepancy between gender and age in the levels of gross incidence of muscular polycystic disease in sheep.

Type		Age	Number of samples	Gross Injuries	%	
Sheep	Male	>18	146	1	0.68 %	2.73%
		< 18	47	4	8.51%	
	Female	>18	136	7	5.14 %	11.7%
		< 18	86	19	22.09%	
Statistical analysis	Chi-square: 37.466 Degrees of freedom3: p-value: 0.000008 (4e-8) * There are significant differences under the significance level (P<0.05)					

Microscopic examination

The results of the microscopic examination of the current study showed all 415 sheep samples, where the percentage of infection was recorded on the basis of three diagnostic methods:
(Pepsin digestion, capillary endoscopy, garlic extract), and there were slight differences in the results of the examinations, as the results of the examination appeared by the method of pepsin digestion 94%, capillary endoscopy 86%, garlic press method 92%. The results of the microscopic examination confirmed the efficiency of the first two tests, and no significant differences were recorded under the significance level (P < 0.05) between the diagnostic methods mentioned in Table (5).

Table 5: shows the percentages of microscopic examination using different detection methods to know their efficiency

Examination method	Number of samples	Gross Injuries	%
Digestive pepsin	415	395	95.18 %
Garlic press machine	100	92	92 %
Slide Smash	100	87	87 %
statistical analysis	Chi-square: 2.374 Degrees of freedom:2 p-value:0.3051353 * There are no significant differences at the level of significance (P < 0.05).		

The relationship of microscopic injury with age

The results of the current study showed that there is no significant difference below the level (P<0.05) between the different age stages of slaughtered sheep. The highest percentages of microscopic infection were recorded in ages above 18 months, which was the highest among the percentages, reaching 96.99%, and ages under 18 months amounted to 94.32%. as in table (6)

Table 6: shows the percentage and numbers of microscopic infections of Sarcocystosis in slaughtered animals divided according to the age of the sheep

Type	Age	Number of samples	Gross Injuries	%
Sheep	under 18 months	282	266	94.32 %

	Over 18 months old	133	129	96.99 %
	total summation	415	395	95.18 %
statistical analysis	Chi-square: 1.4 Degrees of freedom:2 p-value:0.43 * There are no significant differences at the level of significance (P < 0.05).			

Relationship between microscopic infection and sex

The results of the study showed that there were no significant differences under the level of significance (P<0.05), as the value of chi-square X2 = 0.609 was recorded, and the degree of moral difference reached, P = 0.737, so the number of female injuries was 213 out of 222 heads of female sheep, with a percentage of 95.94%, which is the highest rates of injuries during the study period when compared with sex. As for the number of male sheep injuries, it was 182 out of 193 male sheep, at a rate of 94.30%. As shown in Table (7)

Table 7: shows the division of microscopic injuries based on the sex of animals and percentages of sheep.

Type	Age	Number of samples	Gross Injuries	%
Sheep	Male	193	182	94.30 %
	Female	222	213	95.94 %
Total sheep		415	395	95.18 %
Statistical analysis		Chi-square: 0.609 Degrees of freedom:2 p-value:0.737 * There are no significant differences at the level of significance (P < 0.05).		

Assessment of age-sex relationships in microscopic injury

Sheep were divided into groups according to gender and age according to microscopic injuries to find the relationships between the variables and they were as follows:

Under the age of 18 months

The infection rate in male sheep was recorded at 93.15%.
The infection rate in female sheep was recorded at 95.58%.

Over the age of 18 months

Male sheep recorded an infection rate of 97.87%. It is the highest rate of injuries.
Female sheep recorded an infection rate of 96.51%. As in Table (8).

Table 8: shows the percentages of discrepancy between sex and age in the levels of microscopic infection of myoclonic disease in sheep**.**

Type		Age	Number of samples	Gross Injuries	%
Sheep	Male	>18	146	136	93.15 %
		< 18	47	46	97.87 %
	Female	>18	136	130	95.58 %
		< 18	86	83	96.51%
Statistical analysis	Chi-square: 2.525 Degrees of freedom:3 p-value:0.470 * There are no significant differences at the level of significance (P < 0.05).				

Discussion

The current study found that it is difficult to diagnose Sarcocystosis in Sheep before slaughter (Abdullah, 2021) [2]**.** and there is also difficulty in examining the whole carcass by specialists and veterinarians in slaughterhouses. (Al-Hyali *et al*., 2011) [4]. Most Sarcocystis species that infect sheep are specific species of the intermediate host (Bittencourt *et al*., 2016) [11]. Sheep are the mediating host of the most famous Sarcocystis species in Iraq, *S. tenella, S. gigantea and S. medusiformis*, (DE *et al*., 2017) [14]. These species are transmitted by cats (Shnawa & Swar, 2021) [42]. These species are transmitted by cats. A large number of infections with macroscopic cysts indicates the presence of cats in sheep's places in a large way, it shows the main risk factor for infection and because of feeding cats feeding on meat from sheep carcasses Watching cats mainly see the spread of Sarcocystosis (Messages, 2014) [31]. Lots of numbers at the same time Either by ingesting a (high dose) of Sarcocystis eggs or spore sacs at the same time or in a short exposure time with grass contaminated with the grass (Fayer *et al*., 2015) [23]. As for other macroscopic infections due to the age of the animal

(El-Dakhly *et al*., 2011) [18]. The study confirmed the high incidence of infection among the older age groups, depending on the age factor (Gareh *et al*., 2020) [26]. this allows the opportunity for the parasite to develop inside the host's body, as the study has proven with macroscopic cyst infections there is a large disparity between the infections of females and males (Farhang-Pajuh *et al*., 2014) [20]. This is due to the hormonal changes that females go through during pregnancy, Also, no special tendencies of the parasite towards a particular sex were recorded. (Mounika *et al*., 2018) [34].

References

1. Abdel-Ghaffar F, Mehlhorn H, Bashtar AR, Al-Rasheid K, Sakran T, El-Fayoumi H. Life cycle of Sarcocystis camelicanis infecting the camel (Camelus dromedarius) and the dog (Canis familiaris), light and electron microscopic study. Parasitology Research,2009:106(1):189-195. https://doi.org/10.1007/s00436-009-1648-x
2. Abdullah SH. Investigation of Sarcocystis spp. In slaughtered cattle and sheep by peptic digestion and histological examination in Sulaimani Province, Iraq. Veterinary World,2021:14(2):468-474. https://doi.org/10.14202/vetworld., 2021, 468-474.
3. Abubakar S, Teoh B, Hooi P, Italiano CM, Omar SFS, Wong K, *et al*. Outbreak of Human Infection with.,2013:19(12):2012-2014.
4. Al-Hyali NS, Kennany ER, Khalil LY. Fate of macrosarcocyst of sarcocystis gigantea in sheep. Iraqi Journal of Veterinary Sciences,2011:25(2):87-91. https://doi.org/10.33899/ijvs.2011.5652
5. AL-Masuady. Epidemiological & Serological Study of Sarcocystosis to Ovine in Karbala Province A. A Thesis Submitted to the Council of the College of Veterinary Medicine of University of Baghdad in Partial Fulfillment of the Requirements for the Degree of Master Science in Veterinary Medicine –Parasitology By, 2009.
6. El-Morsey A, El-Seify M, Desouky ARY, Abdel-Aziz MM, Sakai H, Yanai T. Morphologic identification of a new Sarcocystis sp. in the common moorhen (Gallinula chloropus) (Aves: Gruiformes: Rallidae) from Brolos Lake, Egypt. Parasitology research,2014:113: 391-397.
7. Al-Saadi SAM, Al-Mussawi KAM, Muhammed. Molecular identification of sarcocystis species infection in sheep in karbala governorate–iraq. Medico-Legal Update,2020:20(1):889-895. https://doi.org/10.37506/v20/il/2020/mlu/194708.
8. Alhayali NS, Hasan MH, Al-Mallah KH. Natural heavy infection with immature sarcocysts of sarcocytis spp. In sheep in mosul city: A case report. Iraqi Journal of Veterinary Sciences,2020:34(2):373-376. https://doi.org/10.33899/ijvs.2019.125994.1210
9. Anemia AF. Molecular and Microscopic Investigation of Sarcocystis Species Isolated from Sheep Muscles in Iran Journal of Food Quality, 2021. https://doi.org/10.1155/2021/5562517
10. Bamac OE, Rogers KH, Arranz-Solís D, Saeij JPJ, Lewis S, Duerr R, *et al*. Protozoal encephalitis associated with Sarcocystis calchasi and S. falcatula during an epizootic involving Brandt's cormorants (Phalacrocorax penicillatus) in coastal Southern California, USA. International Journal for Parasitology: Parasites and Wildlife, 2020, 185-191. https://doi.org/10.1016/j.ijppaw.2020.06.005.
11. Bittencourt MV, Meneses IDS, Ribeiro-Andrade M, de Jesus RF, de Araújo FR, Gondim LFP. Sarcocystis spp. in sheep and goats: frequency of infection and species identification by morphological, ultrastructural, and molecular tests in Bahia, Brazil. Parasitology Research,2016:115(4):1683-1689. https://doi.org/10.1007/s00436-016-4909-5
12. Buxton D. Protozoan infections (Toxoplasma gondii, Neospora caninum and Sarcocystis spp.) in sheep and goats: Recent advances. Veterinary Research,1998:29(3-4):289-310.
13. Collins GH, Sutton RH, Charleston WAG. Studies in Sarcocystis species V: A species infecting dogs and goats; observation on the pathology and serology of experimental Sarcocystosis in goats The New Zealand Veterinary Association.,1980:28:156-158.
14. DE H, SS A, MS B, MG A, YZ O. Molecular identification of Sarcocystis species in imported frozen beef in Egypt. Alexandria Journal of Veterinary Sciences,2017:53(2):72. https://doi.org/10.5455/ajvs.244449
15. Dubey JP, Calero-Bernal R, Rosenthal BM, Speer CA, Fayer R. Sarcocystosis of animals and humans, second edition. In Sarcocystosis of Animals and Humans, Second Edition., 2015. https://doi.org/10.1201/b19184
16. Dubey JP, Calero-Bernal R, Verma SK, Mowery JD. Pathology, immunohistochemistry, and ultrastructural findings associated with neurological sarcocystosis in cattle. Veterinary Parasitology,2016:223:147-152. https://doi.org/10.1016/j.vetpar.2016.04.025
17. Dubey JP, Rosenthal BM. Sarcocystis capracanis-associated encephalitis in sheep. Veterinary Parasitology,2013:197(1-2):407-408. https://doi.org/10.1016/j.vetpar.2013.04.027
18. El-Dakhly KM, El-Nesr KA, El-Nahass ES, Hirata A, Sakai H, Yanai T. Prevalence and distribution patterns of Sarcocystis spp. in buffaloes in Beni-Suef, Egypt. Tropical Animal Health and Production,2011:43(8):1549-1554. https://doi.org/10.1007/s11250-011-9840-2
19. Elmishmishy B. Molecular Characterization of Sarcocystis species in sheep (Issue July)., 2017. https://doi.org/10.13140/RG.2.2.35216.51201

20. Farhang-Pajuh F, Yakhchali M, Mardani K. Molecular determination of abundance of infection with Sarcocystis species in slaughtered sheep of Urmia, Iran. Veterinary Research Forum: An International Quarterly Journal,2014:5(3):181-186. http://www.ncbi.nlm.nih.gov/pubmed/25568716%0Ahttp://www.pubmedcentral.nih.gov/articleren der.fcgi?artid=PMC4279651
21. Fayer R. Sarcocystis spp. in human infections. Clinical Microbiology Reviews,2004a:17(4):894-902. https://doi.org/10.1128/CMR.17.4.894-902.2004
22. Fayer R. spp. in Human Infections. Clin. Microbiol. Rev.,2004b:17(4):894-902. https://doi.org/10.1128/CMR.17.4.894
23. Fayer R, Esposito DH, Dubey JP. Human infections with Sarcocystis species. Clinical Microbiology Reviews,2015:28(2):295-311. https://doi.org/10.1128/CMR.00113-14
24. Florin-Christensen M, Schnittger L. Parasitic protozoa of farm animals and pets. Parasitic Protozoa of Farm Animals and Pets, 2018, 1-438. https://doi.org/10.1007/978-3-319-70132-5
25. Franssen F, Gerard C, Cozma-Petruț A, Vieira-Pinto M, Jambrak AR, Rowan N, *et al*. Inactivation of parasite transmission stages: Efficacy of treatments on food of animal origin. Trends in Food Science and Technology,2019:83:114-128. https://doi.org/10.1016/j.tifs.2018.11.009
26. Gareh A, Soliman M, Saleh AA, El-Gohary FA, El-Sherbiny HMM, Mohamed RH, *et al*. Epidemiological and histopathological investigation of sarcocystis spp. in slaughtered dromedary camels (camelus dromedarius) in Egypt. Veterinary Sciences,2020:*7*(4):1-10. https://doi.org/10.3390/vetsci7040162
27. Lakshmi N, Chaitanya PJ, Chandrashekar R, Bhavani NL. World Journal of Pharmaceutical research Pharmaceutical Compounds,2013:2(4):950-958. https://doi.org/10.20959/wjpr20172-7840
28. Lau YL, Chang PY, Tan CT, Fong MY, Mahmud R, Wong KT. Short report: Sarcocystis nesbitti infection in human skeletal muscle: Possible transmission from snakes. American Journal of Tropical Medicine and Hygiene,2014:90(2):361-364. https://doi.org/10.4269/ajtmh.12-0678
29. Lindsay DS, Dubey JP. Neosporosis, Toxoplasmosis, and Sarcocystosis in Ruminants: An Update. In Veterinary Clinics of North America - Food Animal Practice, 2020, 36(1). Elsevier Inc. https://doi.org/10.1016/j.cvfa.2019.11.004
30. Máca O, González-Solís D. Role of three bird species in the life cycle of two Sarcocystis spp. (Apicomplexa, Sarcocystidae) in the Czech Republic. International Journal for Parasitology: Parasites and Wildlife,2022:17:133-137. https://doi.org/10.1016/j.ijppaw.2022.01.002
31. Messages K. Key messages Key messages.,2014:27:1-8.
32. Metwally DM, Al-Damigh MA, Al-Turaiki IM, El-Khadragy MF. Molecular characterization of sarcocystis species isolated from sheep and goats in riyadh, saudi arabia. Animals,2019:9(5):1-9. https://doi.org/10.3390/ani9050256
33. Morsy K, Abdel-Ghaffar F, Bin Dajem S, Abdel-Gaber R, El Gazar F. First molecular characterization and morphological aspects of Sarcocystis fusiformis infecting water buffalo Bubalus bubalis in Egypt. Acta Parasitologica,2018:63(2):333-345. https://doi.org/10.1515/ap-2018-0038
34. Mounika K, Chennuru S, Ravipati V, Tumati SR, Krovvidi S. Studies on prevalence and histomorphology of Sarcocystis species infecting cattle in Andhra Pradesh, India. Journal of Parasitic Diseases,2018:42(1):77-80. https://doi.org/10.1007/s12639-017-0968-5
35. NS A-H, ER K, AF AT. Effect of lysate of Sarcocystis gigantea in rats. Iraqi Journal of Veterinary Sciences,2011:25(2):81-85.
36. Odening K. The present state of species-systematics in Sarcocystis Lankester, 1882 (Protista, Sporozoa, Coccidia). Systematic Parasitology,1998:41(3):209-233. https://doi.org/10.1023/A:1006090232343
37. Pipia AP, Varcasia A, Zidda A, Dessì G, Panzalis R, Tamponi C, *et al*. Cross-sectional investigation on sheep sarcosporidiosis in Sardinia, Italy. Veterinary Parasitology: Regional Studies and Reports,2016:3-4:13-17. https://doi.org/10.1016/j.vprsr.2016.05.004
38. Prakas P, Bea A, Juozaitytė-Ngugu E, Olano I, Villanúa D, Švažas S, *et al*. Molecular identification of Sarcocystis halieti in the muscles of two species of birds of prey from Spain. Parasites and Vectors,2021:14(1):1-8. https://doi.org/10.1186/s13071-021-04921-0
39. Prakas P, Butkauskas D. Protozoan parasites from genus Sarcocystis and their investigations in Lithuania. Ekologija,2012:58(1):45-58. https://doi.org/10.6001/ekologija.v58i1.2349
40. Prakas P, Rudaitytė E, Butkauskas D, Kutkienė L. Sarcocystis entzerothi n. sp. from the European roe deer (Capreolus capreolus). Parasitology Research,2017:116(1):271-279. https://doi.org/10.1007/s00436-016-5288-7
41. Prakas P, Strazdaitė-Žielienė Ž, Januškevičius V, Chiesa F, Baranauskaitė A, Rudaitytė-Lukošienė, *et al*. Molecular identification of four Sarcocystis species in cattle from Lithuania, including S. hominis, and development of a rapid molecular detection method. Parasites and Vectors,2020:13(1):1-9. https://doi.org/10.1186/s13071-020-04473-9

42. Shnawa BH, Swar SO. Sarcocystosis in Meat-Producing Animals: An Updating on the Molecular Characterization. International Journal of Veterinary Science,2021:(1):144-151. https://doi.org/10.47278/book.vpph/2021.0012
43. Sudan V, Shanker D, Kumar R, Sachan A. Pathological studies on bubaline tissues naturally infected with sarcocystis spp. Journal of Veterinary Parasitology,2019:33(1):8-11. https://doi.org/10.5958/0974-0813.2019.00003.2
44. Swar SO, Shnawa BH. Recent advances in molecular characterization of Sarcocystis species in some meat producing animals: an updated review. Asian Journal of Agriculture and Biology,2021:(1):1-10. https://doi.org/10.35495/AJAB.2020.09.502