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Review Article

Recent advances in molecular characterization of *Sarcocystis* species in some meat producing animals: an updated review

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Abstract

Sarcocystosis is a parasitic disease caused by *Sarcocystis* species that infect humans and animals. It is prevalent in small ruminants like sheep and goats worldwide and causing pathogenic impacts that lead to economic losses owing to carcass condemnation, abortion, and death. Recently, several molecular and phylogenetic analyses have been developed to differentiate *Sarcocystis* species including, the 18S rRNA, 28S rRNA, 18S rDNA, and ITS-1 region. In recent years, the mitochondrial cytochrome c oxidase subunit 1 (cox-1) was successfully used for this purpose. The DNA barcoding using the *cox*1 gene is a reliable tool to distinguish and identify the main *Sarcocystis* genotypes. Therefore, several studies confirmed that the *cox*1 gene is a promising DNA marker for studying the genus *Sarcocystis*. The current review aims to highlight the molecular methods that exist for the identification of *Sarcocystis* species. The results showed that the *Sarcocystis* species of sheep and goats were genetically close related and may be considered as sibling strains, as well as the cross-infection may happen among them. Consequently, the host specificity of several *Sarcocystis* species is questionable. The findings additional emphasized that experimental transmission investigations within the proposed definitive host are required to confirm the characteristics and host ranges of the Sarcocystis *spp.* in sheep and goats. The current review represents updated knowledge about molecular discrimination of *Sarcocystis* species in small ruminants by reviewing and analyzing the recent articles in this aspect.

Keywords: *Sarcocystis* species, Small ruminants, Molecular identification, PCR

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Introduction

Sarcocystis is a coccidian parasite related to the phylum Apicomplexa, class Sporozoasida, order Eucoccidiorida, family Sarcocystidae and subfamily Sarcocystina (Levine, 1986; Dubey et al., 2016). *Sarcocystis* was firstly recorded by Friedrich Miescher in 1843 as a white thread-like structure within the skeletal muscular tissue of a deer mouse from his house in Switzerland, therefore, it is called

Miescher`s tubules. The name of *Sarcocystis* derived from Greek, Sarkos means flesh and Kystis meaning bladder, which refers to the encysted stage in mammalian muscular tissue (Dubey et al., 2016). Approximately 196 *Sarcocystis* species are valid, but just 26 of them have a well-known life cycle. *Sarcocystis* have a wide range of animals that can serve as final hosts and the types of animals that can act as intermediate hosts, which distinguish it from *Neospora caninum* and *Toxoplasma gondii* (Dubey et al., 2016; Lindsay and Dubey, 2020). Sarcocystosis is a world-widely distributed protozoan disease that infects different kinds of mammals, birds, and reptiles. Several species of *Sarcocystis* are pathogenic that result in a severe disease, which can lead to abortion and carcasses condemnation in meat-producing animals, also some species are described as zoonotic (Dubey et al., 2015). Small ruminants as meat-producing animals are one of the main divisions of the food supply chain for humans in most countries and infected with various economically significant parasitic diseases, including sarcocystosis and *Eimeria* spp. infection (Latif et al., 1999; Hassanen et al., 2020).

Globally, a high prevalence of sarcocystosis was recorded in sheep such as 96.1, 86.5, 81.5-90, 100 and 95.3% in Brazil, Izmir-Turkey, Saudi Arabia, Iran, and Egypt, respectively (Beyazit et al., 2007; Dehaghi et al., 2013; Al-Quraishy et al., 2014; Elmishmishy et al., 2018; Minuzzi et al., 2019). Also, Morsy et al. (2011) showed that 79.4% of goats were infected with microsarcocysts in Egypt. In Iraq, Latif et al. (1999) recorded a percentage of infection in sheep and goats as 97 and 97.4%, respectively. Also, in the north of Iraq, the percentage of infection with microsarcocysts reached 97% in sheep and 100% in goats (Zangana and Hussein, 2017), whereas the infection with macrosarcosystis recorded as 9.5 and 8.8% in both sheep and goats, respectively (Swar and Shnawa, 2020).

Moreover, Barham et al. (2005) observed 97 and 34% of goats were infected with microcysts and macrocysts respectively, in Al-Sulaimany province-Iraq. Several methods have been developed for the diagnosis of sarcocystosis. These methods can be categorized into the macroscopic and microscopic examination, serology, and molecular. This review aims to address the current situation of the recent publications that achieved various molecular analyses to confirm the characterization of *Sarcocystis* species and to highlight the gap in the information regarding sarcocystosis.

Life cycle stages

The life cycle of *Sarcocystis* remained unidentified till 1972 when some investigations ultra-structurally investigated the gametogenic and oocyst production of *S. falctula* in poultry within in vitro model (Fayer, 1972; Rommel et al., 1972). The life cycle of *Sarcocystis* requires two obligatory prey-predator hosts for its completion, intermediate host and final host, followed one another successively and described as *diheteroxenous* parasite (Odening, 1998) (Figure 1). The events occurring in the life cycle are as follows:

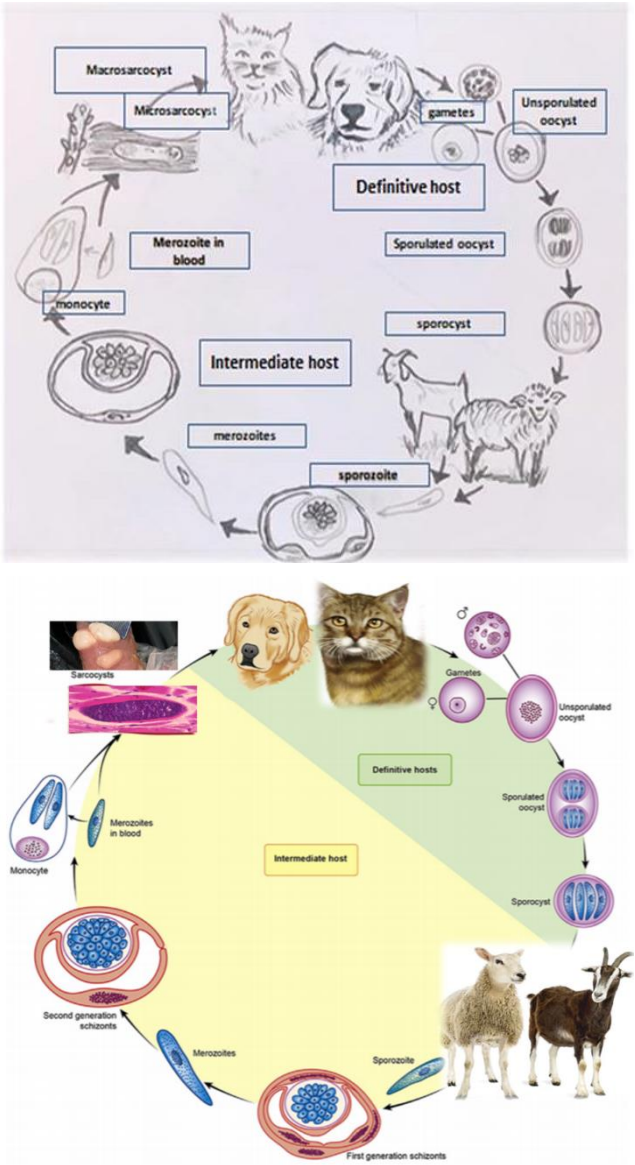

Figure-1: Life cycle of *Sarcocystis* spp. of small ruminants by schematic line- draw (Modified from Lindsay and Dubey, 2020).

Asexual stages

It is found only in the intermediate host, which is mostly a prey animal. Infection begins when *Sarcocystis* oocysts or sporocysts from feces of the final host are ingested by these animals with contaminated food or water. Sporozoites are liberated from the oocysts under the effect of trypsin and bile. These sporozoites invade the gut wall and lodge primarily in the endothelial cells of the small arteries. Four cycles of asexual development, called merogony and schizogony are known (Fayer et al., 2015). During the first three stages of development, sporozoites undergo many nuclear divisions followed by segmentation to generate merozoites, which are motile and crescent in shape. Following these cycles of schizonts, the *Sarcocystis* encysts in the muscles and initially forming metrocytes and then transform to bradyzoites. Sarcocysts having bradyzoites refer to the last encysted stage in the skeletal, cardiac, and smooth muscles of herbivores infected animals, which is infectious for carnivorous animals as definitive hosts (Fayer et al., 2015; Dubey, 2015; Dubey et al., 2016; Khater et al., 2020). Two types of sarcocysts can be found in sheep and goats, including microsarocyst and macrosarcocyst, which related to different species of *Sarcocystis* as shown, in Figure 2 and 3. Till now, there are seven *Sarcocystis spp*. have recorded from domestic sheep, four of them (*S. tenella*, *S. arieticanis*, *S. microps*, and *S. mihoensis*) found to use dogs as definitive hosts. Besides the other three (*S.gigantea*, *S.medusiformis*, and *S. moulei*) that transmitted by felids (Al-Hoot et al., 2005; Kalantari et al., 2016; Gjerde et al., 2020). Goats are intermediate hosts for three common species of *Sarcocystis* (*S. capracanis*, *S. moulei*, and *S.hircicanis*) (Lindsay and Dubey, 2020). Moreover, *S. gigantea* and *S. tenella* species that commonly infect sheep have identified in goats also (Ghaffar et al., 1989; Hong et al., 2016). All the mentioned species of goats were able to form microsarcocysts and utilize dogs as definitive hosts except *S. moulei* and *S gigantea* form macrosarcocysts and use cats as definitive hosts for completing their life cycles. These findings suggest sheep and goats can probably serve as alternative hosts for several closely related *Sarcocystis* spp.

A and B: thick-walled sarcocyst with radial striations of *S. tenella* in sheep esophagi, scale bar= 500 nm. *C:* Heavily infected esophagus of goat showed three different sizes of sarcocysts with inflammation, scale bar = 5 µm. D, E, and F: morphologic features of sarcocysts in the esophagus of goats. Showing a thick wall sarcocysts related to *S. capracanis.* All sections were stained with hematoxylin and eosin. D Scale bar = 500, E= Scale bar = 2 µm, and F Scale bar = 500 nm. (Swar, 2020; unpublished results).

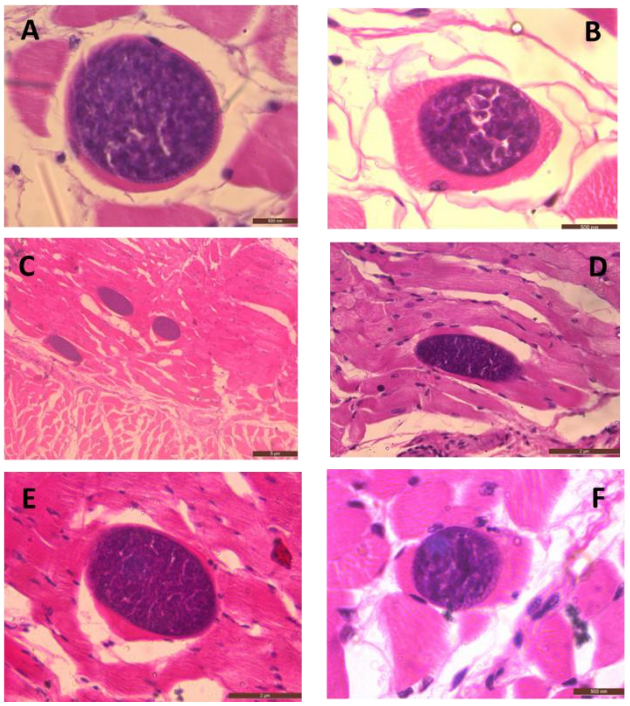

Figure-2: Microscopic sarcocysts in histological section.

Figure-3: Macroscopic sarcocysts in esophagi of sheep and goats (modified from Swar and Shnawa, 2020)

Sexual stages

The final host acquires the infection by ingesting mature sarcocysts within the muscles of infected animals (Lindsay and Dubey, 2020). The Sarcocysts are digested in the digestive system of the definitive host and release the bradyzoites, which invade the mucosa of the small intestine. Then, they are changed into both male and female gametes, namely, microgametes and macrogametes, respectively. These

gametes undergo fertilization to form a zygote, which leads to the formation of the non-motile oocysts. The sexual cycle and fertilization need to be completed within one day. The oocysts of this parasite sporulate in the small intestine. The sporulated oocysts are thin-walled contain two sporocysts, each with four sporozoites. Then, it ruptures, releasing the sporocysts into the intestinal lumen that are excreted with the feces. The prepatent and patent periods differ according to *Sarcocystis spp.*, but in most of them, oocysts are first to shed in the feces after 7 to 14 days post acquiring Sarcocysts (Lindsay and Dubey, 2020).

Species infecting sheep and goats

Several species of Sarcocystis infect sheep (Table 1), some of them transmitted by canids and others by feline (cat). The species transmitted by dogs are mainly pathogenic and cause microsarcocyst in skeletal and cardiac muscles of infected animals. These species, like *S. tenella*, can cause pathologic effects in sheep including, anorexia, anemia, weight loss, abortion, neural symptoms, and death (Dubey et al., 1988; Abdel-Baki et al., 2009). While the species transmitted by cats, for example, *S. gigantea*, and *S. medusiformis* produce macrosarcocyst in the muscular tissue like the esophagus, tongue, and larynx also, they are less pathogenic than the microsarcocysts (Collins et al., 1979; Dubey et al., 2016). There are three common species of this parasite in domestic goats: *S. caprafelis* (synonym *S .moulei*), *S. hiricanis*, and *S. capracanis* (Table1). Commonly, *S. hiricanis* and *S. capracanis* appear as microscopic sarcocysts, whereas *S. moulei* forms macroscopic cysts (Dubey et al., 2016). Clinically, *S. capracanis* is more pathogenic than other species (Collins and Charleston, 1979), the infected goats show fever, weakness, anorexia, weight loss, tremors, abortion, and also lead to death in heavy infection (Dubey et al., 1981).

Nowadays, several publications pointed out that the infection of sheep and goats with species of *Sarcocystis* that are uncommon in these hosts, as the infection of sheep with *S. moulei* that normally infect goats in Saudi Arabia and Iran (Al-Hoot et al., 2005; Kalantari et al., 2016). Also, the infection of goats with *S. gigantea* that commonly infects sheep (Ghaffar et al., 1989) and concluded that the goats can be a host of three species of *Sarcocystis* that are previously classified as *S. moulei*, including *S. ovifelis (S. gigantea)*. Also, Hong et al. (2016) proved the infection of goats with *S. tenella* which is commonly known as sheep specific by molecular and ultrastructural investigation in Korea.

Table-1. Species infecting sheep and goats according to references

Intermediate host	Sarcocystis species	Type of Sarcocyst	Definitive host	Reference
Sheep	*S. tenella* (*S.ovicanis*)	Micro-sarcocyst	Dogs, coyote, red fox	Dubey et al., 2016; Lindsay and Dubey,2020
Sheep	*S. arieticanis*	Micro-Sarcocyst	Dogs	=
Sheep	*S. gigantea* (*S .ovifelis*)	Macro-Sartcocyst	Cats	=
Sheep	*S medusiformis*	Macro-Sarcocyst	Cats	=
Sheep	*S. moulei* (*S.caprafelis*)	Macro-Sarcocyst	Cats	Al-Hoot et al., 2005; Kalantari et al., 2016
Sheep	*S. mihoensis*	Macro-sarcocyst	Dogs	Saito et al.,1997
Sheep	*S. microps*	Micro-sarcocyst	Dogs	Wang et al.,1988
Goats	*S. capracanis*	Micro-Sarcocyst	Dogs, coyote, red fox	Dubey et al.,2016; Lindsay and Dubey,2020
Goats	*S. hircicanis*	Micro-Sarcocyst	Dogs	=
Goats	*S. moulei (S. caprafelis)*	Macro-Sarcocyst	Cats	=
Goats	*S. gigantea* (*S .ovifelis*)	Macro-Sarcocyst	Cats	Ghaffar et al., 1989
Goats	*S.tenella*	Micro-sarcocyst	Dogs	Hong et al., 2016

Molecular diagnosis of *Sarcocystis spp*.

Recently, researchers achieved great success in several molecular techniques for identifying different *Sarcocystis* species that infect livestock animals and are known to be host-specific. Among them, the 18S rRNA, 28S rRNA, 18S r DNA, mitochondrial cytochrome C oxidase subunit 1 gene (cox1), and ITS-1 region (Dubey et al., 2014; Blazejewski et al., 2015; Ng et al., 2015; Hu et al., 2017; El-Morsey et al., 2019) as shown in Table 2.

Table-2. Some recent publications in molecular diagnosis of *Sarcocystis spp* from sheep and goats

***Sarcocystis* species and method**	**Molecular findings**	**References**
Six *Sarcocystis* spp. Ribosomal RNA sequences.	Two groups of *Sarcocystis spp*. were identified molecularly. The first group contains two species that need cats as definitive hosts, and the second one has *Sarcocystis* spp. that the dogs act definitive hosts for them. Also, *Sarcocystis* was separated from *Toxoplasma* and the classification of them as two genera into different subfamilies of the Sarcocystidae was refuted.	Tenter et al.,1992
S. tenella RFLP-PCR genotyping for the 18S rRNA	Twenty- two of 602 Brazilian sheep were positive for *Sarcocystis* species. Identification of the 18S rRNA gene of *S. tenella* (GenBank accession number L24383-1).	da Silva et al., 2009
S. gigantea and *S. tenella* Mitochondrial cytochrome c oxidase subunit I gene (cox1) and the nuclear ssrRNA gene sequences	For the first time established cox1 as a new genetic marker for the identification of *Sarcocystis spp*. Also, it presented the first molecular characterization of *S. gigantea (KC209733)* besides *S. tenella* of sheep in Norway. Results of ssrRNA gene sequences showed that three of the four sequences of microscopic sarcocysts isolated from sheep were indistinguishable (KC209734–KC209736), one nucleotide was incompatible with the fourth sequence (KC209737). Sequence identity in BLAST showed sequences were most similar (99.1% identity) to *S. capracanis* (L76472), whereas had 96.4% similarity with *S. tenella* (L24383).	Gjerde, 2013a
S. tenella proved by 18S r RNA gene sequence with PCR-RFLP technique.	RFLP-PCR analysis explained that microscopic cysts were *S. tenella* in 70% of the tested specimens of sheep from Iran.	Shahbazi et al., 2013
S. gigantea and *S. tenella* in sheep by the 18s rRNA gene sequence	Genotyping of ten sarcocysts revealed that the pattern of macrsarcocyst identified as *S. gigantea* and the microsarcocyst is *S. tenella* in Iranian sheep.	Bahari et al., 2014
S. gigantea and *S. medusiformis* by the PCR and RFLP techniques.	The results proved that fat macrosarcocysts were *S. gigantea* as 29.31% and thin macro-sarcocysts were *S. medusiformis* in 7.52%.	Farhang-Pajuh et al., 2014
S. neurona strain SO SN1 RNA and Genomic DNA Were sequenced	Identified the first genome sequence of *S. neurona.* The accession number of the nucleotide sequence was SRP052925 in the National Center for Biotechnology Information **(http://www.ncbi.nlm.nih.gov/bioproject/252030)**	Blazejewski et al., 2015
S. capracanis by 18S RNA sequences	Microsarcocysts in goats skeletal muscles were characterized as *S. capracanis*	Kutty et al., 2015
S. tenella by 18S r RNA method.	Sequence findings of Sarcocystis spp. in sheep confirmed the presence of *S. tenella* only in Italy.	Bacci et al., 2016
S. arieticanis and *S.capracanis* in sheep and goats respectively by18S ribosomal DNA(r DNA)	Molecular and ultrastructural results proved the first confirmation of *S.tenella* and *S.arieticanis* in sheep and *S.capracanis* in goats in Brazil.	Bittencourt et al., 2016
S. tenella in goats With 18S r DNA sequence	First molecular and ultrastructural documentation of *S.tenella* in domestic goats in Korea	Hong et al., 2016
18S rRNA gene of *S. tenella*	Molecular characterization and phylogeny of *S.tenella* in sheep in Baghdad-Iraq.	Whaeeb and Faraj, 2016

S. gigantea and *S. moulei* in sheep by partial 18S r RNA gene sequences.	Among thirty sequences of DNA sarcocysts, 20 (66.7%), 6 (20%), and 4 (13.3%) specimens were characterized as *S. gigantea*, *S. moulei*, and *Sarcocystis* spp., respectively. The first record of *S. mulei* in sheep, as well as they, concluded that sheep consider as an alternative host for this species besides goats in Iran.	Kalantari et al., 2016
S. gigantea by analysis of part of 18S r RNA gene sequence	The first documentation of *S. gigantea* in sheep from Argentina	Gual et al., 2017
Identification of *S, tenella,* and *S. arieticanis* by the 18S r RNA gene,28S r RNA gene, mitochondrial cox1 gene, and ITS-1 region sequences	Genetic characterization of *S. tenella*, and *S. arieticanis* by four markers in sheep from China.	Hu et al., 2017
18S rRNA gene of *S.tenella* and *S.capracanus.*	First detection and molecular identification of *S.tenella* and *S.capracanus* from sheep and goats in Tunisia	Amairia et al., 2018
18S RNA gene of *S.tenella* from sheep	Recorded *S tenella* from Egypt in the Gene Bank (accession number KP263759.1.)	Hussein et al., 2018
S. tenella and *S. arieticanis* Analysis of cox1 gene sequence in sheep.	First molecular confirmation of *S. tenella* (MH561854) in *Ovis ammon* sheep in China by mitochondrial cox1 gene sequences that consider as an appropriate intermediate host.	Dong et al., 2018
S. tenella and *S. capracanis.* Mitochondrial cox1 sequences	Fist molecular confirmation of *S. tenella* and *S. capracanis*.by cox1 gene in sheep and goats respectively, from Saudi Arabia. As well as, they concluded the strong phylogenetic relation between *Sarcocystis* species from sheep and goats.	Metwally et al., 2019
S. tenella and *S. arieticanis* in sheep. Sequence by 18S r RNA, 28S r RNA, COX1,and ITS-1	The first molecular and ultrastructural confirmation of eleven isolates of *S.tenella* and *S.arieticanis* from sheep in Egypt under the accession numbers MH413034- MH413040 and MH413045- MH413048 in the Gene Bank. They concluded that COX1and ITS-1 genes seemed to be the best genetic markers amongst the other tested. Also, this study mentioned to the *S. tenella* of sheep and S. capracanis the goat's species as closely related sister species.	El-Morsey et al., 2019
S. tenella, S. arieticanis, S. gigantea, S. medusiformis, and *S. mihoensis*-like. Cytochrome c oxidase subunit 1 gene (cox1), 18S RNA, and 28S r RNA gene.	Five species of *Sarcocystis* from sheep were molecularly identified in sheep by three genetic markers. The finding of this study suggested that *S. medusiformis, S.gigantea*, and *S.moulei* have genetically sister sequences. All these three species formed macrosrcocysts in sheep, and utilize the cat as a definitive host for all of them.	Gjerde et al., 2020
S. arieticanis by 18S rRNA gene sequence	First molecular characterization of S. *arieticanis* in cardiac muscles of sheep in Egypt. Sarcocystis cyst size considered as a significant feature in the classification.	Hussein, 2020

The first sequenced genome in the genus *Sarcocystis* was related to *S. neurona.* The genome has 127-Mbp and it is more than double the size of other sequenced coccidian genomes (Blazejewski et al., 2015). Tenter et al. (1992) identified two monophyletic groups of *Sarcocystis* species; one of them represents the species that uses the cat as the definitive host, while the second includes the species that require dogs as final hosts for completing their life cycles.

Initially, *Sarcocystis spp*. were described as largely host-specific; however, over the last few years, numerous *Sarcocystis* species employing different animals as intermediate hosts were confirmed. As a result, host specificity became questionable.

Regarding this aspect, In Saudi Arabia, Al-Hoot et al. (2005) identified *S. moulei* from the sheep by ultra-structural study, the species which commonly infect goats. Also, *S. moulei* was confirmed molecularly in sheep by Kalantari et al. (2016) in Iran. In the same regard, *S. capracanis* was recognized in the cerebrospinal fluid of 2 sheep with meningoencephalitis in the United Kingdom (Formisano et al., 2013). Therefore, sheep can serve as an alternative intermediate host for this species besides goats. In addition, in a study regarding sarcocystosis of sheep in Egypt, Elmishmishy et al. (2018) recorded the complete similarity between the isolate of *S. gigantea* from sheep and that of *S. moulei* and suggested the cross-transmission of *S. moulei* between sheep and goats and they are phylogenetically close. Another study also demonstrated that the *S. tenella* of sheep and *S. capracanis* the goat's species are genetically closely related sister species by ultra-structural and phylogenetic analysis using 18S r RNA, 28S r RNA, COX1, and ITS-1 genetic markers (El-Morsey et al., 2019). In Saudi Arabia, a recent study concluded a strong phylogenetic correlation among *Sarcocystis* species from sheep and goats (Metwally et al., 2019). Yang et al. (2001) confirmed that morphologically identical species from two different intermediate hosts should be considered the same species. However, many *Sarcocystis* species seem to have a wider intermediate host option than previously known. Also, more recent findings suggest that *S. medusiformis*, *S. gigantea*, and *S. moulei* possess genetically sister sequences. All these species proved to form macrosrcocysts in sheep, and the cat is the definitive host for them (Gjerde et al., 2020). This phenomenon has also been observed in the infection of cattle and water buffalo with uncommon *Sarcocystis* spp. As a result, these investigations lead to the suggestion that *Sarcocystis* species are non-specific to the host (Jehle et al., 2009; Xiang et al., 2011; Gjerde et al., 2016; Dakhil et al., 2017; El-kady et al., 2018).

Gjerde (2013a; 2013b) concluded that cox1 sequences appear to explain better than the ssrRNA gene sequences for characterization of the closely related species of *Sarcocystis*, and recommended using this novel genetic marker in future studies. Additionally, Gjerde et al. (2016) suggested that the cox1 gene was superior to both 18S and 28S rRNA genes. In the same regard, El-Morsey et al. (2019) confirmed that cox1and ITS-1 genes looked to be the best genetic markers amongst the other tested in the differentiation of the closely related *Sarcocystis spp.* within the intermediate hosts because of their high divergence, as shown in Table 2. Another investigation compared the new sequences of four genetic markers (18S rRNA, 28S rRNA, mitochondrial *COX1*, and *ITS-1)* for *S. tenella* and *S.arieticanis*, and confirmed that the ITS-1 region could be more useful for distinguishing the closely related *Sarcocystis* spp. owing to its high divergence (Hu et al., 2017). Besides, the same was true for the genetic similarity of sheep and goats infection with *Eimeria* spp., which was recorded by phylogenetic analysis of the ITS-1 sequences of this parasite in Egypt. The sequence of (ITS-1) region of *E. ahsata* was 100% similar to ovine *E. ahsata* and clustered in a single clade with *E. cardinalis* and *E. faurei*. On the other hand, *E. arloingi* was 100% identical to *E. arloingi* of goat and clustered with bovine *E. ellipsoidalis* (Hassanen et al., 2020).

Conclusion

In conclusion, the information about *Sarcocystis spp.* is still not highly clarifying. However, several publications related to the biological aspect of Sarcocystosis have been achieved, whereas there are numerous ongoing researches on molecular and ultrastructural diagnosis still perform globally. The 18S rRNA ribosomal gene and mitochondrial cytochrome c oxidase subunit I (cox1) genes were the most analysis used for molecular characterization and phylogenetic analysis of *Sarcocystis* spp. The results of some reviewed articles showed that the *Sarcocystis* species of sheep and goats were closely related and may consider as sibling strains, as well as the cross-infection, may happen among these animals. Therefore, the host specificity of several *Sarcocystis* species is questionable. The findings further emphasize that experimental transmission investigations within the proposed definitive host are required to confirm the characteristics and host ranges of the *Sarcocystis spp.* within these two ruminants.

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References

Abdel-Baki AA, Allam G, Sakran T and El-Malah EM, 2009. Lambs infected with UV-attenuated

sporocysts of *Sarcocystis ovicanis* produced abnormal sarcocysts and induced protective immunity against a challenge infection. Korean J. Parasitol. 47: 131-138.

Al-Hoot AS, Al-Qureishy SA, Al-Rasheid K and Bashtar AR, 2005. Microscopic study on *Sarcocystis moulei* from sheep and goats in Saudi Arabia. J. Egypt. Soc. Parasitol. 35: 295-312.

Al-Quraishy S, Morsy K, Bashtar AR, Ghaffar FA and Mehlhorn H, 2014. *Sarcocystis arieticanis* (Apicomplexa: Sarcocystidae) infecting the heart muscles of the domestic sheep, Ovis aries (Artiodactyla: Bovidae), from K. S. A. on the basis of light and electron microscopic data. Parasitol. Res. 113: 3823–3831.

Amairia S, Amdouni Y, Rouatbi M, Rjeibi MR, Awadi S and Gharbi M, 2018. First detection and molecular identification of *Sarcocystis spp*. in small ruminants in North-West Tunisia. Transbound Emerg. Dis. 65: 441–446.

Bacci C, Vismarra A, Passeri B, Sciarrone F, Mangia C, Genchi M, Fabbi M, Vicari N, Bruini I, Brindani F and Kramer L, 2016. Detection of *Toxoplasma gondii* and *Sarcocystis tenella* in indigenous CornigLiese sheep in Italy using serological and molecular methods. Small Rum. Res. 135: 13-16.

Bahari P, Salehi M, Seydabadi M and Mohammadi A, 2014. Molecular identification of macroscopic and microscopic cysts of *sarcocystis* in sheep in north Khorasan province, Iran. J. Mol. Cellul. Med. 3: 51-6.

Barham M, Stutzer H, Karanis P, Latif BM and Neiss WF, 2005. Seasonal variation in *sarcocystis species* infections in goat in northern Iraq. Parasitol. 130: 151-156.

Beyazit A, Yazicioğlu O and Karaer Z, 2007. The prevalence of ovine *Sarcocystis species* in Izmir province in sheep, Ankara Univ. Vet. Fak. Derg. 54:111-116.

Bittencourt MV, Meneses IDS, Ribeiro-Andrade M, de Jesus RF, de Araújo FR and Gondim LFP, 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: 1683–1689.

Blazejewski T, Nursimulu N, Pszenny V, Dangoudoubiyam S, Namasivayam S, Chiasson M A, Chessman K, Tonkin M and Swapna LS, 2015. Systems-Based analysis of the *Sarcocystis neurona* genome identifies pathways that contribute to a heteroxenous life cycle. mBio. 6: 1-16.

Collins GH, Atkinson E and Charleston WAG, 1979. Studies on *Sarcocystis* species III: the macrocystic species of sheep. New Zealand Vet. J. 27: 204-206.

Collins GH and Charleston WAG, 1979. Studies on *Sarcocystis species* IV: A species infecting dogs and goats; development in goats. New Zealand Vet. J. 27: 260-262.

Dakhil HG, Abdallah BH and Abdallah FA, 2017. Molecular identification of *sarcocystis fusiformis* and *S. moulei* infecting water buffaloes (*bubalus bubalis*) in southern Iraq. World J. Pharm. Res. 6: 215-229.

Da Silva RC, Su C and Langoni H, 2009. First identification of *Sarcocystis tenella* (Railliet, 1886) Moulé, 1886 (Protozoa: Apicomplexa) by PCR in naturally infected sheep from Brazil. Vet. Parasitol. 165: 332–336.

Dehaghi MM, Fallahi M, Masoud S and Radfar MH, 2013. Survey of *Sarcocystis* infection in slaughtered sheep in Kerman Abattoir, Kerman, Iran. Comp. Clin. Pathol. 22: 343–346.

Dong H, Su R, Wang Y Zhang L, Yang Y and Hu J, 2018. *Sarcocystis species* in wild and domestic sheep (Ovis ammon and Ovis aries) from China. BMC Vet. Res. 14: 377.

Dubey JP, Weisbrode SE, Speer CA and Sharma SP, 1981. Sarcocystosis in goats: clinical signs and pathologic and hematologic findings. J. Am. Vet. Med. Assoc. 178: 683-699.

Dubey JP, Lindsay DS, Speer CA, Fayer R and Livingston CW, 1988. *Sarcocystis arieticanis* and other *Sarcocystis* species in sheep in the United States. J. Parasitol. 74: 1033-1038.

Dubey JP, 2015. Foodborne and waterborne zoonotic *sarcocystosis.* Food Waterborne Parasitol. 1: 2–11.

Dubey JP, Calero BR, Rosenthal BM, Speer CA and Fayer R, 2016. Sarcocystosis of animals and humans. 2nd Ed. Boca Raton: CRC Press; Taylor and Francis Group.

Dubey JP, Lane EP and van Wilpe E, 2014. *Sarcocystis cafferin. sp.* (Protozoa: Apicomplexa) from the African Buffalo (Syncerus caffer). J. Parasitol. 100: 817–827.

Dubey JP, van Wilpe E, Calero-Bernal R, Verma SK and Fayer R, 2015. *Sarcocystis heydorni* n. sp. (Apicomplexa: Sarcocystidae) with cattle (Bos taurus) and human (Homo sapiens) cycle. Parasitol. Res. 114: 4143–4147.

El-kady AM, Hussein NM and Hassan AA, 2018. First molecular characterization of *Sarcocystis spp*. in cattle in Qena Governorate, Upper Egypt. J. Parasit. Dis. 42: 114-121.

Elmishmishy B, Al-Araby M, Abbas I and Abu-Elwafa S, 2018. Genetic variability within isolates of *Sarcocystis species* infecting sheep from Egypt. Vet. Parasitol: Reg. Stud. Rep. 13: 193–197.

El-Morsey A, Abdo W, Sultan K, Elhawary NM and AbouZaid AA, 2019. Ultrastructural and Molecular Identification of the sarcocysts of *Sarcocystis tenella* and *Sarcocystis arieticanis* Infecting Domestic Sheep (*Ovisaries*) from Egypt. Acta Parasitologica. 64: 501–513.

Farhang-Pajuh F, Yakhchali M and Mardani K, 2014. Molecular determination of abundance of infection with *Sarcocystis* species in slaughtered sheep of Urmia, Iran. Vet. Res. Forum. 5: 181–186.

Fayer R, 1972. Gametogony of *Sarcocystis* species in cell culture. Sci. 175: 65-67.

Fayer R, Esposito DH and Dubey JP, 2015. Human Infections with *Sarcocystis Species*. Clin. Microbiol. Rev. 28: 295–311.

Formisano P, Aldridge B, Alony Y, Beekhuis L, Davies E, Del Pozo J, Dunn K, English K, Morrison L, Sargison N, Seguino A, Summers BA, Wilson D, Milne E and Beard PM, 2013. Identification of *Sarcocystis capracanis* in cerebrospinal fluid from sheep with neurological disease. Vet. Parasitol. 193: 252–255.

Ghaffar FA, Heydorn AO and Mehlhorn H, 1989. The fine structure of cysts of *Sarcocystis moulei* from goats. Parasitol. Res. 75: 416–418.

Gjerde B, 2013a. Phylogenetic relationships among *Sarcocystis* species in cervids, cattle and sheep inferred from the mitochondrial cytochrome c oxidase subunit I gene. Int J Parasite. 43: 579–591.

Gjerde B, 2013b. *Sarcocystis* species in red deer revisited: with a re-description of two known species as *Sarcocystis elongatan*. sp. and *Sarcocystis truncata* n. sp. based on mitochondrial sequences. Parasitol. 141: 441–452.

Gjerde B, Hilali M and Abbas IE, 2016. Molecular differentiation of *Sarcocystis buffalonis* and *Sarcocystis levinei* in water buffaloes (*Bubalus bubalis*) from *Sarcocystis hirsuta* and *Sarcocystis cruzi* in cattle (Bos taurus). Parasitol. Res. 115: 2459–2471.

Gjerde B, de la Fuente C, Alunda JM and Luzón M, 2020. Molecular characterization of five *Sarcocystis* species in domestic sheep (Ovis aries) from Spain. Parasitol. Res. 119: 215-231.

Gual l, Bartiley PM, Katzer F, Innes EA, Canton G J and Moore DP, 2017. Molecular confirmation of *Sarcocystis gigantea* in a naturally infected sheep in Argentina: A case report. Vet. Parasitol. 248: 25-27.

Hassanen EAA, Anter RGA, El-Neshwy WM and Elsohaby I, 2020. Prevalence and phylogenetic analysis of *Eimeria* species in sheep and goats in Sharkia Governorate, Egypt. Pak. Vet. J. http://dx.doi.org/10.29261/pakvetj/2020.064

Hong EJ, Sim C, Chae JS, Kim HC, Park J, Choi KS, Yu DH, Park CH, Yoo JG and Park BK, 2016. Ultrastructural and molecular identification of *Sarcocystis tenella* (Protozoa, Apicomplexa) in naturally infected Korean native goats. Vet. Med. 61: 374–381.

Hu JJ, Huang S, Wen T, Esch GW, Esch GW, Liang Y and Li HL, 2017. *Sarcocystis* spp. in domestic sheep in Kunming City, China: prevalence, morphology, and molecular characteristics. Parasitol. 24: 30.

Hussein NM, Hassan AA and Abd Ella OH, 2018. Morphological, ultrastructural, and molecular characterization of *Sarcocystis tenella* from sheep in Qena governorate, upper Egypt. Egypt. Acad. J. Biol. Sci. 10: 11-19.

Hussein NM, 2020. Morphological and molecular characterization of *Sarcocystis arieticanis* from the heart muscles of domestic sheep, *Ovis aries*, in Qena, upper Egypt. J. Adv. Vet. Res. 10: 73-80.

Jehle C, Dinkel A, Sander A, Morent M, Romig T, Luc PV, De TV, Thai VV and Mackenstedt U, 2009. Diagnosis of *Sarcocystis* spp. in cattle (Bos taurus) and water buffalo (*Bubalus bubalis*) in Northern Vietnam. Vet. Parasitol. 166: 314–320.

Kalantari N, Khaksar M, Ghaffari S and Hamidekish SM, 2016. Molecular Analysis of *Sarcocystis Spp*. Isolated from Sheep (*Ovis aries*) in Babol area, Mazandaran Province, Northern Iran. Iran. J. Parasitol. 11:73–80.

Khater HF, Ziam H, Abbas A, Abbas RZ, Raza MA, Hussain K, Younis EZ, Radwan T and Selim A, 2020. Avian coccidiosis: Recent advances in alternative control strategies and vaccine development. Agrobiol. Records. 1: 11-25.

Kutty MK, Latif B, Muslim A, Hussaini J, Daher AM, Heo CC and Abdullah S, 2015. Detection of sarcocystosis in goats in Malaysia by light

microscopy, histology, and PCR. Trop. Anim. Health Prod. 47: 751–756.

Latif B, Al-Delemi J, Mohammed B, Al-Bayati S and Al-Amiry A, 1999. Prevalence of *Sarcocystis spp* in meat producing animals in Iraq. Vet. Parasitol. 84: 85-90.

Levine ND, 1986.The Taxonomy of *Sarcocystis* (Protozoa, Apicomplexa) Species. J. Parasitol. 72:372.

Lindsay DS and Dubey JP, 2020. Neosporosis, Toxoplasmosis, and Sarcocystosis in ruminants: An update. Vet. Clin. Food Anim. 36: 205–222.

Metwally DM, Al-Damigh MA, Al-Turaiki IM and El-Khadragy MF, 2019. Molecular characterization of *Sarcocystis* species isolated from sheep and goats in Riyadh, Saudi Arabia. Anim. 9: 256.

Minuzzi CE, Cezar AS, Bräunig P, Portella LP, Rodrigues F de S, Sangioni LA and Vogel FSF, 2019. Occurrence of *Sarcocystis gigantea* macrocysts and high frequency of *S. tenella* microcysts in sheep from southern Brazil. Vet. Parasitol. Reg. Reports. 15: 100256.

Morsy K, Saleh A, Al-Ghamdi A, Abdel-Ghaffara F, Al-Rasheid K, Bashtar A-R, Al Quraishy S and Mehlhorn H, 2011. Prevalence pattern and biology of *Sarcocystis capracanis* infection in the Egyptian goats: A light and ultrastructural study. Vet. Parasitol. 181: 75-82.

Ng YH, Fong MY, Subramaniam V Shahari S and Lau YL, 2015. Short communication: Genetic variants of *Sarcocystis cruzi* in infected Malaysian cattle based on 18S rDNA. Res. Vet. Sci. 103: 201–204.

Odening K, 1998. The present state of species-systematics in *Sarcocystis* Lankester, 1882 (Protista, Sporozoa, Coccidia). Syst. Parasitol. 41: 209-233.

Rommel M, Heydron AO and Gruber F, 1972. Contribution on the life cycle of Sarcocporidia I. the sporocyst of *Sarcocystis tenella* in the feces of the cat. Berl. Munich TierztlWochenscher. 85: 101-105.

Saito M, Shibata Y, Kubo M and Itagaki H, 1997. *Sarcocystis mihoensis n. sp*. from sheep in Japan. J. Vet. Med. Sci. 59: 103–106.

Shahbazi A, Asfaram S, Fallah E, Khanmohammadi M, Nematollahi A and Moharami T, 2013. Identification of *Sarcocystis tenella* and *Sarcocystis arieticanis* isolated from slaughtered sheep in Tabriz abattoir using parasitological and PCR-RFLP methods. J. Comp. Pathobiol. Iran. 10: 959-964.

Swar SO and Shnawa BH, 2020. Prevalence and Microscopic Studies of Mixed *Sarcocystis* Infection in Naturally Infected Sheep and Goats in Soran City, Erbil- Iraq. J. Res. Lepid. 51: 669-684.

Swar SO, 2020 Molecular and ultrastructural characterization of *Sarcocystis species* in domestic sheep and goats from Soran city, Erbil-Iraq.Unpublished PhD Thesis. Department of Biology, Faculty of Science, Soran University, Iraq.

Tenter AM, Baverstock PR and Johnson AM, 1992. Phylogenetic relationships of *Sarcocystis* species from sheep, goats, cattle and mice based on ribosomal RNA sequences. Int. J. Parasitol. 22: 503-513.

Wang G, Wei T, Wang X, Li W, Zhang P, Dong M and Xiao H, 1988. The morphology and life cycle of *Sarcocystis microps n.* sp. in sheep of Qinghai in China. China Vet. Technol. 6: 9-11.

Whaeeb ST and Faraj AA, 2016. Molecular Identification and phylogeny of Microscopic *Sarcocystis* Sheep in Baghdad Province. Int J. Adv. Res. Biol. Sci. 3:50-56.

Xiang Z, He Y, Zhao H, Rosenthal BM, Dunams DB, Li X, Zuo Y, Feng G, Cui L and Yang Z, 2011. *Sarcocystis cruzi*: Comparative studies confirm natural infections of buffaloes. Exp. Parasitol. 127: 460-466.

Yang Z, Zuo Y, Yao YG, Chen XW, Yang GC and Zhang YP, 2001. Analysis of the 18S rRNA genes of *Sarcocystis* species suggests that the morphologically similar organisms from cattle and water buffalo should be considered the same species. Mol. Biochem. Parasitol. 115: 283-288.

Zangana IK and Hussein SN, 2017.Prevalence of Sarcocystis Species (*Sarcocystis ovicanis* and *Sarcocystis capricanis*) in Tongue Muscle of Sheep and Goats in Duhok Province, Kurdistan Region, North Iraq. ARO Sci. J. Koya Univ. 1: 36-40.

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Swar SO: Literature review and wrote the first draft of the manuscript
Shnawa BH: Conceived idea, manuscript final writing, editing and approval