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SARCOCYSTOSIS IN MEAT-PRODUCING ANIMALS: AN UPDATING ON THE MOLECULAR CHARACTERIZATION

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INTRODUCTION

Sarcocystosis is a globally distributed zoonotic protozoan disease that infects a wide variety of mammals, reptiles, and birds. This disease is caused by *Sarcocystis* species, which are a coccidian intracellular protozoan parasite of the genus *Sarcocystis* that belongs to the family Sarcocystidae of the phylum Apicomplexa (Fayer et al. 2015). About 200 valid species of *Sarcocystis* have been identified, which vary in their pathogenicity to the host, ranging from avirulent to highly virulent; some species are zoonotic; however, complete life cycles are known only for 26 species (Dubey 2015; Dubey et al. 2016).
This parasite has an obligatory two-host life cycle (prey and predator) known as intermediate and definite hosts. The asexual part of its life cycle is completed in the intermediate host, which is usually a herbivore animal. At the same time, sexual stages of its development occur in the definite or final host, a carnivore or omnivore animal (Lindsay and Dubey 2020). *Sarcocystis* is non-pathogenic in the definite host, and several species are also non-pathogenic in the intermediate hosts. In general, species transmitted by the canid's definite host are pathogenic, while those transmitted by the felid's definite host are non-pathogenic (Lindsay and Dubey 2020).

Human infection with Sarcocystis spp. is relatively rare. Still, humans serve as definite or intermediate hosts for this parasite. They become the final host by consuming under-cooked beef or pork meat, infected with *S. homonis* and *S. suihominis*, respectively. Symptoms of human intestinal *Sarcocystis* infection include nausea, abdominal discomfort, stomach ache, and diarrhea (Fayer et al. 2015). Humans can become an intermediate or aberrant host for seven or more *Sarcocystis* species, including *S. nesbitti*, which was identified in humans through18SrDNA sequence analysis. *S. nesbitti* infection is considered as a new zoonotic disease caused by the ingestion of food or water contaminated with this *Sarcocystis* species. Moreover, *S. heydorni,* which mostly infects cattle, is also considered as a zoonotic protozoan (Dubey 2015; Dubey et al. 2016). Infection with *Sarcocystis* protozoan in the intermediate host can lead to morbidity, mortality, abortions, decreased meat production, and increased meat condemnation due to presence of macrosarcocysts. Up to now, the importance of human sarcocystosis has failed to gain necessary attention regardless of the potential for muscular or intestinal Sarcocystis infection to infect a considerable number of people (Fayer 2004; Tappe et al. 2013).
Sarcocysts caused by sarcocystosis are common in many domestic and wild animals and can cause mortality in these infected animals. There are two types of *Sarcocystis* cysts, the microscopic and the macroscopic sarcocysts (Dubey 2015).
Sarcocystosis is also considered to be one of the most prevalent intracellular protozoan diseases of livestock (Fayer 2004). Animals including cattle, goats, and sheep are susceptible to sarcocystosis (Dubey and Lindsay 2006) and are considered as intermediate hosts. Cysts are only formed within the muscles of the intermediate hosts. Therefore, they are called sarcocysts, which commonly indicate a wide-ranging hosts and worldwide distribution (Abdel-Ghaffar *et al*., 2009). Several species of *Sarcocystis* develop macroscopic sarcocysts in the tissue of domesticated animals. Amongthese, S. *caprafelis* (syn. *S. moulei*) is found in goats, while *S. gigantea* (syn. *S. ovifelis*) and *S. medusiformis* in sheep (Dubey and Lindsay 2006).Similarly, *S. hirsuta* and *S. hominis* form macroscopic sarcocysts in cattle, while *S. buffalonis and S. fusiformis* form these cysts in the water buffalo (Lindsay and Dubey 2020).

History of Sarcocystosis

In 1843, milky white threads in the muscles of a deer mouse, *Mus muscula,* were observed by Friedrich Miescher in Switzerland (Miescher 1843). These threads were known as Miescher's tubules for many years. Later, the causative organism of the problem was named as *Sarcocystis* after Lankester (1882), a Greek word derived from “sarkos” that means muscle or flesh, and “kystis” meaning bladder, describing the parasite's form that encysted in the tissue of the intermediate hosts (Fayer 2004). Thereafter, *S. muris* was the first species named in the genus *Sarcocystis* by the Swiss scientist Friedrich Miescher. The house mouse was the only known intermediate host for this organism (Ruiz and Frenkel 1976). In 1967, crescent-shaped structures typically found in cultures of sarcocystiswere investigated and the organism was identified as a protozoan, a close relative of *Toxoplasma* spp. and *Eimeria* spp. (Fayer 2004).
In 1969, Mandour identified a new *Sarcocystis* species in rhesus macaques named *S. nesbitti*, after Mr. Nesbitt, who

observed the trophozoites in stained smears. The definite hosts of *S. nesbitti* are now-a-days known to be snakes, whereas several primates, including human beings, can be intermediate hosts (Mandour 1969).

Scientists debated whether the *Sarcocystis* spp. were protozoa until 1967, after the first Sarcocystis report when the spindle or crescent-shaped bodies (bradyzoites) in the sarcocystis were studied by electron microscopy. Its organelles resembled to other apicomplexan protozoa, such as *Toxoplasma* and *Eimeria* (Fayer 2004).

The terminology for *Sarcocystis* spp. was suggested by combining the Latin names of both intermediate and definite hosts, such as *Sarcocystis ovicanis (S. tenella)*, for which sheep is an intermediate host, while the dog acts as a final host.Similarly, *S. ovifelis (S. gigantea)* in which sheep serves as an intermediate host, while the cat is the final host. *S. bovicanis* comes from two Latin words: *bos* for cattle and *canis* for dog (Mehlhorn 2016). Some historical landmarks concerning *Sarcocystis* are listed in Table 1.

Classification of *Sarcocystis* species

According to the classification system proposed by Levine (1986), *Sarcocystis* parasite is classified as:

Phylum Apicomplexa	Levine (1970)
Class Sporozoasida	Leuckart (1879)
Subclass Coccidiasina	Leuckart (1879)
Order Eucoccidiorida	Leger and Duboscq (1910)
Suborder Eimeriorina	Leger (1911)
Family Sarcocystidae	Poche (1913)
Subfamily Sarcocystinae	Poche (1913)
Genus *Sarcocystis*	Lankester (1882)

Life cycle of *Sarcocystis* species

The life cycle of *Sarcocystis* species was unknown until 1972, when some investigators ultrastructurally studied the gametogonic and oocyst formation properties of *S. falctula* in poultry during *in vitro* experiments (Fayer 1972; Rommel et al. 1972). In 1973, Wallance (1973) fed experimental mice with coccidia collected from the feces of a naturally infected cat and thus, he induced sarcocysts formation in mice.

The life cycle of *Sarcocystis* species needs two obligatory prey-predator hosts for its completion, intermediate host and definite host, followed by one another sequentially and designated as a di- heterogeneous parasite (Odening 1998) (Fig. 1). The stages occurring during the life cycle of *Sarcocystis* species are as follows:

Asexual stages

The asexual stages of *Sacocystis* parasite development occur only in the intermediate host, which is generally a prey animal. The infection starts when these animals ingest *Sacocystis* oocysts or sporocysts from the food or water contaminated with feces of the final host. Sporozoites are released from the ingested sporocysts by the action of trypsin and bile. The free sporozoites invade the gut wall and lodge themselvesin the endothelial cells of the small arteries. Four cycles of asexual reproduction are distinguished. Several nuclear divisions occur in the sporozoites, followed by segmentation to generate merozoites, which are motile and crescent-shaped. Following these schizonts' cycles, the *Sarcocystis* encysts itself in the muscles and form metrocytes, which are changed to bradyzoites. Sarcocyst having bradyzoites indicates the last encysted stage in the skeletal, cardiac, and smooth muscles of infected herbivores, infectious for carnivorous animals as definite hosts (Dubey 2015; Fayer et al. 2015; Dubey et al. 2016).

Two types of tissue sarcocysts can occur in sheep, goats, and cattle; either as microscopically or macroscopically visible structures of different *Sarcocystis spp*, as shown in Fig. 2 and Fig. 3. Fig. 4 illustrates the Banana-shaped bradyzoites prepared by muscle mincing and squash methods of fresh esophageal tissue from infected sheep and goats and stained with Giemsa stain.

Sexual stages

A definite host acquires the infection by ingesting mature sarcocysts from muscles of an infected animal (Lindsay and Dubey 2020). These sarcocysts are digested in the digestive system of the definite host and release the bradyzoites, which invade mucosa of the small intestine. Then, they are transformed into male and female gametes, called microgametes and macrogametes, respectively. After fertilization, these gametes form a zygote, which develops into the non-motile oocysts. The oocysts sporulate in the small intestine. The sporulated oocysts are thin-walled, each having two sporocysts: Each sporocyst contains four sporozoites. Then, it ruptures, liberating the sporocysts into the lumen of intestine; these sporocysts are defecated with feces (Lindsay and Dubey 2020), as has been shown in Fig. 1.

Ultrastructure of *Sarcocystis* spp

Sarcocystis spp. are single-cell eukaryotic organisms that contain a nucleus, nucleolus, endoplasmic reticulum, ribosomes, Golgi apparatus, and mitochondria. They also have the characteristic apicomplexan organelles, such as the apical rings (conoidal rings), polar ring, a conoid, pellicle, subpellicular microtubules, micropores, rhoptries, and micronomes, as shown in Fig. 5 (Ghaffar et al. 1989; Al-Quraishy et al. 2014; Dubey et al. 2016).

The intermediate host becomes infected by ingesting the sporocysts, the latter release sporozoites in the small intestine. These sporozoites appear in the mesenteric arteries and lymph nodes, where they eventually liberate merozoites into the blood, initiating the development of the sarcocysts in muscle. Resultantly, bradyzoites areformed, which is known as the infective stage for the consumer (Markus et al. 2004).

A sarcocyst plays a significant role in the transmission, as well as in the taxonomy, of *Sarcocystis* spp. It results from the invasion of merozoite into a myocyte or neural cell. Then the merozoite becomes rounded to change into a

metrocyte, with several organelles of the apical complex, like the micronemes, conoid and apical rings disappear. In contrast, ribosomes, endoplasmic reticulum and mitochondria become more abundant, and the nucleus becomes larger (Dubey et al. 2016). Within the cysts, the parasite multiplies by endodyogony or endopolygony, leading to the formation of metrocytes and later thousands of infectious cystozoites (bradyzoites), as shown in Fig. 6.

Sarcocystis species that are infecting ruminants

Sheep, goats and cattle are ungulates, 'hooved' animals that are members of the Order Artiodactyla (animals with cloven hooves), suborder Ruminantia (ruminants or cud-chewing animals), and Family Bovidae. These animals are herbivores, and they meet all their glucose requirements from gluconeogenesis. The subfamily Capra includes sheep and goats (Underwood et al. 2015).
Numerous spp. of *Sarcocystis* infect sheep, some of them are transmitted via canids and others by feline (cat). The species transmitted by dogs are mostly pathogenic and produce microsarcocyst in the skeletal and cardiac muscles of the animal. These species, like *S. tenella,* can lead to pathological effects in sheep, like anorexia, anemia, weight loss, abortion, neural symptoms, and even death (Dubey et al. 1988; Abdel-Baki et al. 2009). However, the species transmitted by cats, for instance *S. gigantea* and *S. medusiformis,* form macrosarcocyst in the esophagus, tongue, and larynx; the pathological effects of macrosarcocyst are more severe than those of the microsarcocysts (Collins et al. 1979; Dubey et al. 2016).

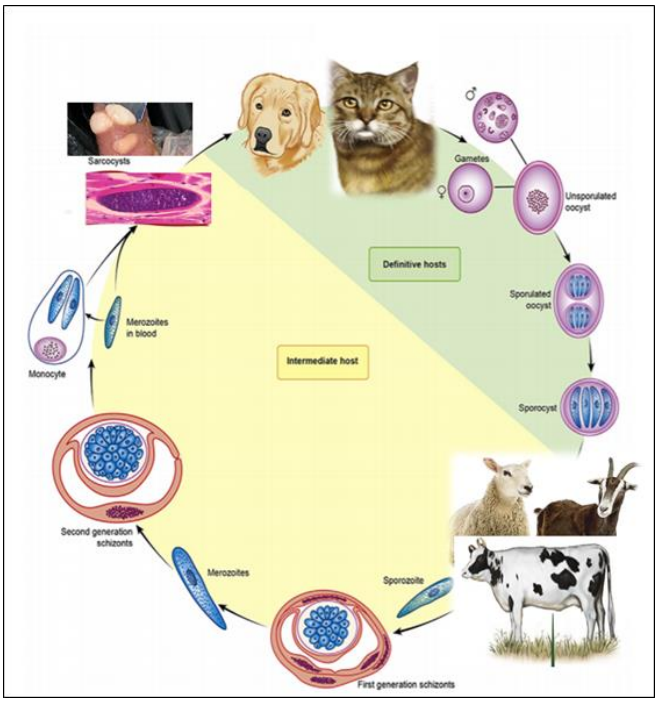

Fig. 1: The life cycle of *Sarcocystis* spp. from ruminants (Modified from Lindsay and Dubey 2020; Swar and Shnawa 2021).

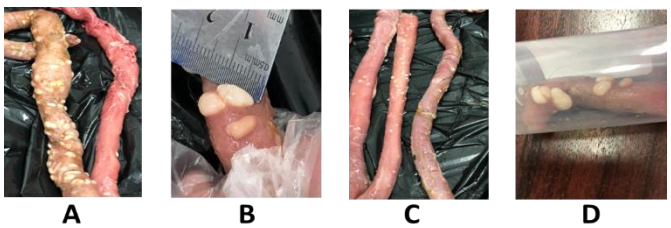

Fig. 2: Appearance of macroscopic sarcocysts in the esophagi of goats (A&B) and sheep (C&D) (Swar and Shnawa 2021). Note several cysts appear with different sizes and shapes on the surface of infected esophagus.

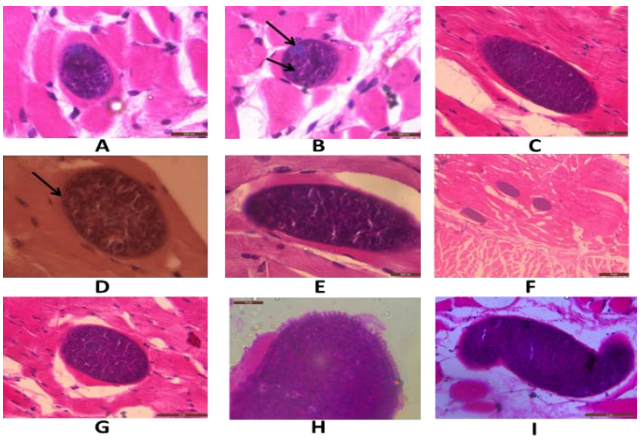

Fig. 3: Microscopic sarcocysts within the esophagi of infected sheep and goats (Swar 2021).

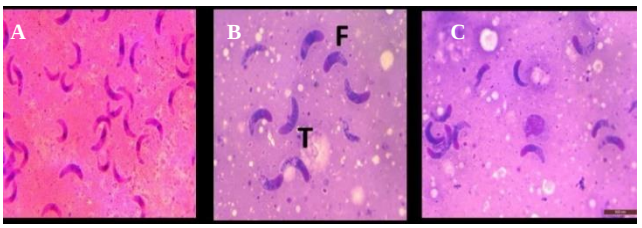

Fig. 4: Banana-shaped bradyzoites by muscle mincing and squash technique of fresh esophageal tissue from sheep and goats stained with Giemsa stain (A, B, and C). Bradyzoites from macroscopic sarcocyst of goats and sheep. B also shows fat (F) and thin (T) types of bradyzoites. Scale bar =500nm. (Swar 2021).

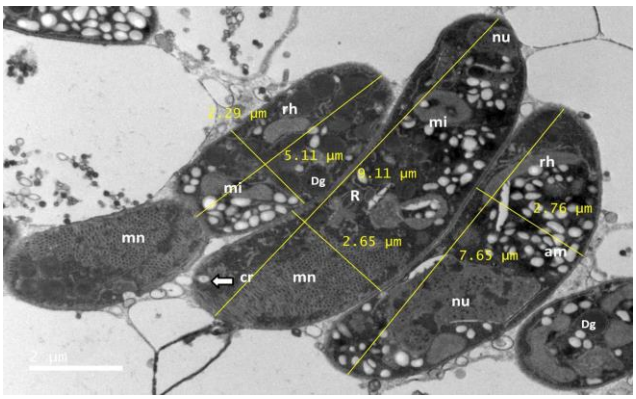

Fig. 5: Transmission electron micrograph of a longitudinal section of macroscopic sarcocyst of *Sarcocystis* spp. of sheep shows bradyzoites. Note conoidal ring (cr), amylopectin (am), numerous micronemes (mn), subterminal nucleus (nu), rhoptries (rh), dense granules as black structure (white arrow), amylopectin as white granules (am), ribosome(R) and mitochondrion (mi). Scale bar= 2 µm. (Swar and Shnawa 2020).

Three *Sarcocystis* species have been identified in domestic goats; these include *S. caprafelis* (synonym *S. moulei*), *S. hiricanis*, and *S. capracanis*. The *S. hiricanis* and *S. capracanis* are usually associated with microscopic sarcocysts, whereas *S. moulei* produces macroscopic cysts (Dubey et al. 2016). Medically, *S. capracanis* shows more severe pathological effects than those of the other two species (Collins and Charleston 1979). The infected goats may show fever, anorexia, weight loss, tremors, abortion, and death in severe cases (Dubey et al. 1981). However, some investigations documented the infection of sheep and goats with Sarcocystis species that are unusual in these hosts, such as the infection of sheep with *S. moulei* that commonly infects goats in Saudi Arabia (Al-Hoot et al. 2005) and Iran (Kalantari et al. 2016). Similarly, *S. gigantean*, which usually infects sheep, can also cause infection in goats (Ghaffar et al. 1989) and it was suggested that goats can be a host for three species of Sarcocystis described as *S. moulei*, including *S. ovifelis (S. gigantea)*. Moreover, using molecular and ultrastructural techniques, Hong et al. (2016) evidenced the infection of Korean goats with *S. tenella*, which is commonly known as sheep specific.

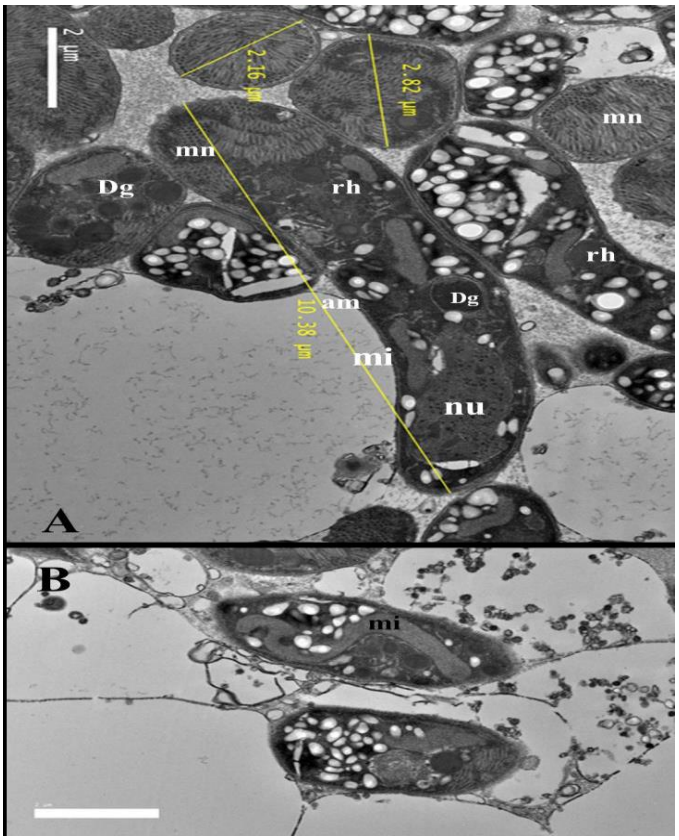

Fig. 6: Transmission electron micrographs of macroscopic sarcocyst of *Sarcocystis spp* from sheep: (A). Longitudinal and cross-sections of bradyzoites showing conoidal ring (cr), amylopectin (am), micronemes (mn), nucleus (nu), and dense granules (dg), and mitochondrion. Scale bar= 2 µm. (B). Cross-section of bradyzoite with one long twisted mitochondrion (mi), nucleus (nu), dense granules (dg), and amylopectin as white granules (am). The parasite is surrounding by a membrane of parasitophorous vacuole within the host cell cytoplasm-scale bar= 2 µm for both micrographs (Swar 2021).

Five species of Sarcocystis have been identified in cattle: these include *Sarcocystis cruzi*, *Sarcocystis heydorni*, *Sarcocystis hirsuta*, *Sarcocystis hominis*, and *Sarcocystis rommeli*; the definite hosts for these species are canines (*S. cruzi*), felines (*S. hirsuta*, *S. rommeli*), and primates (*S. heydorni*, *S. hominis*).
Generally, four *Sarcocystis* species can infect water buffalo, including *S. buffalonis*, *S. dubeyi*, *S. fusiformis*, and *S. levinei*.

Molecular Characterization of *Sarcocystis spp*

In recent years, researchers gained outstanding achievements in several molecular procedures to identify various *Sarcocystis* spp that can infect different animals and are recognized as host-specific. These molecular procedures include 18S rRNA, 28S rRNA, 18S rDNA, 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).
The first genetically identified species of the genus *Sarcocystis* through genome sequence was the *S. neurona*. Its genome contains 127-Mbp, and its size is twice that of the size of other coccidian genomes (Blazejewski et al. 2015). Tenter et al. (1992) recognized two monophyletic groups of *Sarcocystis* spp. One group represents the species for which cats arethe definite hosts. In contrast, the second group has the species that need dogs as definite hosts in their life cycles.
Previously, *Sarcocystis* spp. were known to be primarily host-specific, but, during the last few years, a large number of *Sarcocystis* spp which use different animals as intermediate hosts were identified. Consequently, host specificity for various *Sarcocystis* spp. is questionable. In this regard, Al-Hoot et al. (2005) characterized ultrastructurally the *S. moulei* in sheep infection, the species which usually infects goats in Saudi Arabia. Moreover, in Iran, *S. moulei* was documented genetically in sheep (Kalantari et al. 2016). Similarly, *S. capracanis* was identified from the cerebrospinal fluid of sheep suffering from meningoencephalitis in the United Kingdom (Formisano et al. 2013). Based on these findings it can be suggested that sheep can act as an alternative intermediate host for these species in addition to goats. In a study regarding *Sarcocystis* infection from Egypt, Elmishmishy et al. (2018) documented the resemblance of *S. gigantea* from sheep with *S. moulei* from goats. They proposed the cross-transmission of *S. moulei* amongst sheep and goats, and suggested them to be phylogenetically related. Other researchers confirmed the genetic similarity between *S. tenella* of sheep and *S. capracanis* from goats and considered them as sister species (El-Morsey et al. 2019).
Moreover, Saudi Arabian researchers also revealed the presence of phylogenetic association among *Sarcocystis* spp from both hosts, sheep and goats (Metwally et al. 2019). According to Yang et al. (2001), morphologically identical species from two different intermediate hosts should be classified as similar species. However, many *Sarcocystis* spp. seem to have a more comprehensive intermediate host opportunity than previously recognized.

Table 1: Historical landmarks regarding *Sarcocystis,* modified from Dubey et al. *(*2016)

Scientist's name	Year	The outcomes
Miescher	1843	*Sarcocystis* reported from the muscle of house mouse
Lankester	1882	The genus *Sarcocystis* named
Fayer	1972	Sexual stages cultured in vitro
Rommel et al.	1972	Two obligatory hosts required for completion life-cycle
Fayer & Johnson	1973	The pathogenicity proved with recognition of vascular phase
Heydorn	1975	Several *Sarcocystis* species are recognized within a given host.
Fayer & Johnson	1975	Chemotherapy (Amprolium) is documented
Fayer et al.	1976	Abortion results from *Sarcocystis* infection in cows
Dubey et al.	1981	Protective immunity against sarcocystosis is proved in goats
Dubey et al.	1989	Classification of *Sarcocystis* species depending on cyst wall morphology is recommended.
Dubey et al.	1991	First detection of the causative agent of equine protozoan myeloencephalitis and classified *as Sarcocystis neurona.*
Blazejewski et al.	2015	First characterization of *Sarcocystis* genome that related to *S.neurona.*

Table 2: Several recent articles concerning the molecular characterization of *Sarcocystis* spp. of meat-producing animals.

Sarcocystis spp.	Molecular technique	Host	Country	Reference
S. tenella	RFLP-PCR for the 18S rRNA	Sheep	Brazil	(da Silva et al. 2009)
S. fusiformis,S.cruzi, S. homonis, S. hirsuta	RFLP-PCR for 18S rDNA	Cattle & Water buffalo	Vietnam	(Jehle et al. 2009)
S. gigantea and *S. tenella.*	Mitochondrial cytochrome c oxidase subunit I gene (cox1) and the nuclear ssr RNA	Norwegian Sheep Argentinean cattle	Norway	(Gjerde 2013a)
S. hirsuta and *S. sinensis*				
S. tenella	18S r RNA gene sequence with PCR-RFLP	Sheep	Iran	(Shahbazi et al. 2013)
S. cafferi	18S Rrna& COX1	Buffalo	South Africa	(Dubey et al. 2014)
S. gigantea and S. tenella	18S rRNA	Sheep	Iran	(Bahari et al. 2014)
S. gigantea and *S. medusiformis*	18S rRNA	Sheep	Iran	(Farhang-Pajuh et al. 2014)
S. cruzi	18S rDNA	Cattle	Malaysian	(Ng et al. 2015)
S. capracanis	18S rRNA	Goats	Malaysia	(Kutty et al. 2015)
S. tenella	18S rRNA	Sheep	Italy	(Bacci et al. 2016)
S. arieticanis & *S. capracanis*	18S rDNA	Sheep	Brazil	(Bittencourt et al. 2016)
S. tenella	18S rDNA	Goats	Korea	(Hong et al. 2016)
S. tenella	18S rRNA	Sheep	Iraq	(Whaeeb and Faraj 2016)
S. gigantea & *S. moulei*	18S rRNA	Sheep	Iran	(Kalantari et al. 2016)
S. gigantea	18S rRNA	Sheep	Argentina	(Gual et al. 2017)
S. fusiformis & S. moulei	18S rRNA	Water buffaloes	Iraq	(Dakhil et al. 2017)
S. tenella & S. arieticanis	18S r RNA gene,28S r RNA, cox1, and ITS-1 region	Sheep	China	(Hu et al. 2017)
S. tenella & S. capracanis	18S rRNA	Sheep &goats	Tunisia	(Amairia et al. 2018)
S. tenella	18S rRNA	Sheep	Egypt	(Hussein et al. 2018)
S. tenella and *S. arieticanis*	cox1	Sheep	China	(Dong et al. 2018)
S. gigantea& S. tenella	18S rRNA	Sheep	Egypt	(Elmishmishy et al. 2018)
S. fusiformis	18S rRNA&cox1	Buffaloes	China	(Mei Ren et al. 2019)
S. cruzi & S. hjorti	18S rRNA	Cattle	Egypt	(El-kady et al. 2018)
S. cruzi	18S rRNA	Cattle	Korea	(Choi et al. 2018)
S. tenella & S. capracanis	Cox1	Sheep & goats	Saudi Arabia	(Metwally et al. 2019)
S. bovifelis	Cytochrome C Oxidase subunit I mitochondrial (mtDNA COI) gene	Cattle	Italy	(Rubiola et al. 2019)
S. tenella and *S. arieticanis*.	18S rRNA, 28S rRNA, cox1 and ITS-1	Sheep	Egypt	(El-Morsey et al.2019)
S. arieticanis, S. tenella, S. gigantea, S. medusiformis, & S. mihoensis	Cox1,18S and 28S rRNA	Sheep	Spain	(Gjerde et al. 2020)
S. arieticanis	18S rRNA	Sheep	Egypt	(Hussein et al.2020)
Sarcocystis spp.	18SrRNA	Sheep	Iraq	(Al-Saadi et al. 2020)
S. gigantea, S. moulei, & S. medusiformis	18S rRNA	Sheep & goats	Iraq	(Swar and Shnawa, 2020)

More recent genetic findings propose that *S. medusiformis*, *S. gigantea* and *S. moulei* have identical sister genome sequences. These species are documented to form macrosrcocysts in sheep, while cats serve asthe final host for them (Gjerde et al. 2020). This fact has also been detected in the cattle and water buffalo with uncommon *Sarcocystis* spp infection., Results of several other studies also suggest that *Sarcocystis* spp. are non-specific for the intermediate 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) proved that the sequences of COX1 are better than the ssRNA gene for identifying the closely associated spp of *Sarcocystis* and recommended it as a

novel bio- genetic marker for further studies. Gjerde et al. (2016) recommended COX1 as a superior gene to 18S and 28S rRNA genes as a bio-genetic marker. In the same aspect, El-Morsey et al. (2019) proved the priority of COX1 and ITS-1 genes as the best genetic markers compared to other genes to differentiate the closely related *Sarcocystis* spp owing to the high divergence among them. Another study evaluated the sequences of 4 genetic markers (18S rRNA, 28S rRNA, mitochondrial *COX1* and *ITS-1)* specific for *S. tenella* and *S. arieticanis*.

It has been confirmed that the ITS-1 region is more helpful for distinguishing the closely related *Sarcocystis* spp. because of their high divergence (Hu et al. 2017). The same was true for the genetic resemblance between sheep and goats infection with *Eimeria* spp., which was phylogenetically confirmed by the ITS-1 sequences technique in Egypt. The sequence of ITS-1 gene of *E. ahsata* was 100% identical with *E. ahsata* and clustered in one clade with *E. cardinalis* and *E. faurei*. Alternatively, the similarity was 100% between *E. arloingi* and *E. arloingi* of goats. It is clustered with *E. ellipsoidalis* of bovine sources (Hassanen et al. 2020). Conventionally, the Sarcocystis' ultrastructural features are considered as fundamentals for the identification of numerous *Sarcocystis* spp. within the same intermediate host (Hu et al. 2017; Huang et al. 2019). Currently, genomic sequence analysis is an essential technique to clarify whether morphologically similar *Sarcocystis* spp. from different intermediate hosts are the same species or not. Findings of several recent studies concerning the molecular characterization of *Sarcocystis* spp isolated from meat-producing animals have been summarized in Table 2.

Conclusion

Recently, researchers achieved outstanding success in molecular aspects to identify various *Sarcocystis* spp that infect livestock. Various molecular techniques evaluated for this purpose are 18S rRNA, 28S rRNA, 18S rDNA, mitochondrial cytochrome C oxidase subunit 1 gene (COX1), and ITS-1 region. Usually, the Sarcocystis' ultrastructural features are considered as fundamentals for the identification of many *Sarcocystis* spp. within the same intermediate host. At present, genomic sequence analysis is necessary to clarify whether morphologically similar *Sarcocystis* from different intermediate hosts are the same species or not. *Sarcocystis* spp. are known as primarily host-specific, but a large number of Sarcocystis spp using different animals as intermediate hosts were identified during the last few years. As a result, the issue of host specificity of *Sarcocystis* species remains questionable.

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