Sarcocystis

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Abstract

Sarcocystis (derived from the Greek words *sarx*, which means flesh, and *kystis*, which means bladder) are apicomplexan protozoans that cause sarcocystosis or sarcocystiosis. Infections are characterized by the formation of numerous sarcocysts, which are essentially parasite-full sacs ranging in size from micrometers to several centimeters, in the muscles or nervous tissue of a great variety of animals. The genus is composed of more than 100 species that differ in pathogenicity, host specificity, and sarcocyst structure and location. *Sarcocystis* are obligatory intracellular, with a typical coccidian life cycle, consisting of merogony, gametogony, and sporogony. The life cycle involves an intermediate and a definitive host, usually an herbivore and a carnivore, respectively. At first, a series of asexual reproduction steps culminate with sarcocyst formation. Ingestion of cyst-infected tissues by the definitive host leads to sexual reproduction of the parasite in the digestive tract, followed by excretion of infective forms in the feces. The cycle is closed when an intermediate host becomes infected by the fecal-oral route. Most *Sarcocystis* are species-specific for intermediate and family-specific for definitive hosts. Infection of farm animals is sometimes associated with the reduction in quality and quantity of meat, wool, and fiber, resulting in important economic losses. Additionally, some *Sarcocystis* species are zoonotic. Thus, the study of sarcocystosis constitutes an active field of research.

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4.1 Morphology, Life Cycle, and Host-Pathogen Interactions

Sarcocystis was first reported in Switzerland in 1843 by Miescher, who found white threads in the skeletal muscle of a house mouse—*Mus musculus*—which came to be known as tubules of Miescher (Levine 1986). Until the 1970s, the taxonomic position of the group was not clear, and the major criteria for naming new species were cyst structure and host species. However, studies based on intermediate host specificity indicated that some *Sarcocystis* parasites with structurally similar cysts are actually different species, for example, *S. tenella* and *S. capracanis*. Additionally, some species of *Sarcocystis*, such as *S. falcatula* and *S. neurona*, can infect numerous host species (Dubey 2015). This information, as well as sequencing data, is currently helping to change the status of some species and rename others.

Some of the most important species of *Sarcocystis* that affect farm animals and pets are shown in Table 4.1. Even though sarcocystosis is mainly a veterinary problem, some species are pathogenic to man, such as *S. hominis* and *S. suihominis*. Humans are the definitive hosts of these parasites and become infected by the ingestion of raw or undercooked meat of cattle and pig, respectively, which act as intermediate hosts. Humans can also serve as accidental intermediate or aberrant hosts for several species of *Sarcocystis*, through the ingestion of oocysts (Fayer 2004).

Table 4.1 *Sarcocystis* species in farm animals and pets

Intermediate host	*Sarcocystis* species	Definitive host	References
Pig (*Sus scrofa*)	*S. miescheriana*	Dog (*Canis familiaris*), raccoon (*Procyon lotor*), wolf (*Canis lupus*), red fox (*Vulpes vulpes*), and jackal (*Canis aureus*)	Golubkov et al. (1974), Dubey (2015) and Meshkov (1980)
	S. suihominis	Human (*Homo sapiens*) and primates (*Macaca mulatta*, *Macaca irus*, *Pan troglodytes*, and *Papio cynocephalus*)	
	S. porcifelis	Cat (*Felis catus*)	
Cattle (*Bos taurus*)	*S. cruzi*	Dog (*Canis familiaris*), coyote (*Canis latrans*), red fox (*Vulpes vulpes*), crab-eating fox (*Cerdocyon thous*), raccoon dog (*Procyon lotor*, *Nyctereutes procyonoides*), and wolf (*Canis lupus*)	Bucca et al. (2011), Dubey et al. (1989), Dubey (2015), Gjerde (2016), Nourani et al. (2010), Nourollahi Fard et al. (2009), Rodrigues et al. (2008), Saito et al. (1994) and Soulsby (1987)
	S. hirsuta	Cat (*Felis catus*)	
	S. hominis	Human (*Homo sapiens*), rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*), baboon (*Papio cynocephalus*), and possibly chimpanzee (*Pan troglodytes*)	
	S. rommeli	Unknown	

Table 4.1 (continued)

Intermediate host	*Sarcocystis* species	Definitive host	References
Sheep (*Ovis aries*)	*S. tenella*	Dog (*Canis familiaris*), coyote (*Canis latrans*), and red fox (*Vulpes vulpes*)	Dubey et al. (1989), Dubey (2015), Levine (1986) and Munday and Obendorf (1984)
	S. arieticanis	Dog (*Canis familiaris*)	
	S. mihoensis		
	S. microps		
	S. gigantea	Cat (*Felis catus*)	
	S. medusiformis		
Goat (*Capra hircus*)	*S. capracanis*	Dog (*Canis familiaris*), coyote (*Canis latrans*), red fox (*Vulpes vulpes*), and crab-eating fox (*Cerdocyon thous*)	Dubey (2015) and Levine (1986)
	S. hircicanis	Dog (*Canis familiaris*)	
	S. moulei	Cat (*Felis catus*)	
Equine (*Equus spp.*)	*S. betrami*	Dog (*Canis familiaris*)	Heydorn et al. (1975), Levine (1986), Soulsby (1987) and Dubey (2015)
	S. fayeri		
	S. equicanis		
	S. neurona	Opossum (*Didelphis virginiana*, *Didelphis albiventris*)	
South American camelids	*S. aucheniae*	Dog (*Canis familiaris*)	Carletti et al. (2013), Martín et al. (2016) and Moré et al. (2016)
	S. masoni	Unknown	
Dog (*Canis familiaris*)	*S. caninum*	Unknown	Dubey (2015)
	S. svanai		
Cat (*Felis catus*)	*S. felis*	Unknown	
Chicken (*Gallus gallus*)	*S. wenzeli*	Dog (*Canis familiaris*) and cat (*Felis catus*)	Dubey (2015) and Mansfield et al. (2001)
	S. horvathi	Unknown	

4.1.1 Morphology

Sarcocystis parasites undergo numerous morphological changes that allow distinct functions necessary to carry out host cell invasion, asexual multiplication, or sexual reproduction along their life cycle. Stages that undergo asexual multiplication are *schizonts* and *metrocytes*; host cell invasion is carried out by *merozoites, bradyzoites*, and *sporozoites*, while sexual reproduction involves *micro-* and *macrogametes* produced by *micro-* and *macrogamonts* and leads to the formation of *oocysts*. Importantly, sporozoites and bradyzoites are produced in wall-enclosed structures containing multiple parasites—the *sporocyst* and the *sarcocyst*—which guarantee an efficient transmission to the intermediate and definitive host, respectively.

The main features of *Sarcocystis* stages and structures are described below. As a rule, the phylum-characteristic apical complex is clearly observed in infective stages that interact with and internalize in host cells, while it is not apparent in stages engaged in asexual or sexual multiplication.

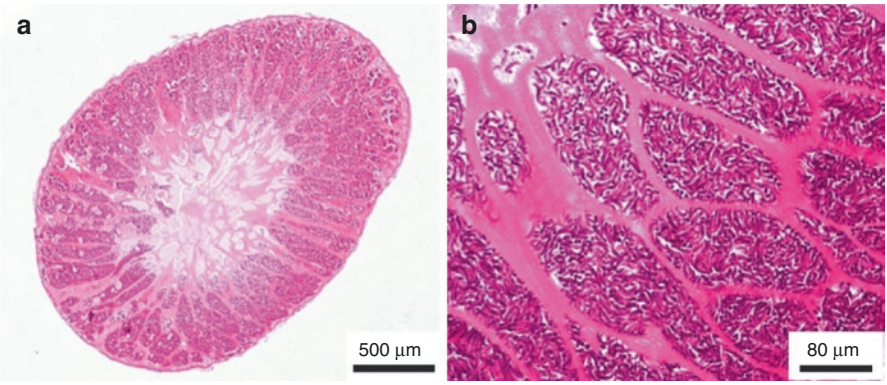

Fig. 4.1 Cross-section of a *Sarcocystis aucheniae* sarcocyst dyed with hematoxylin-eosin. Cytophaneres directed to the center (**a**), 4×; sectioned part of a cyst (**b**), 40×; thousands of bradyzoites arranged in compartments enclosed by thick septa

The schizont is the first parasite stage found in the intermediary host. It develops after a sporozoite has invaded an intermediary host cell, which is generally, but not exclusively, an endothelial cell of a mesenteric lymph node. Early schizonts are ovoid and contain a large nucleus and a single nucleolus. In a process known as endopolygeny, the nucleus gets lobulated and shows several nucleoli. A spindle apparatus with microtubules and two centromeres is associated to each lobe. It guides the genetic material to the lobe end, where a merozoite is formed, and eventually buds, giving the schizont the appearance of a rosette of merozoites. Schizonts develop free in the host cell cytoplasm and are not contained in a parasitophorous vacuole (PV).

Merozoites disseminate the infection in the intermediary host. They are motile, crescent-shaped organisms, with a rhoptry-less apical complex. After budding from a schizont, they can be found free in the blood or located within mononuclear cells. In the latter case, they can divide to form two merozoites by endodyogeny. Upon invasion of a suitable host cell, they start a new schizogony cycle (Fayer 2004).

Sarcocysts are the most characteristic structures produced by *Sarcocystis* parasites. They constitute the last stage of the asexual phase in the intermediary host and are generated after a merozoite has invaded a myocyte or a nervous cell. The membrane of the PV that encloses the parasite and the material underlying it form a wall, providing a safe microenvironment for multiplication. According to the species, sarcocysts can be found in skeletal, cardiac, or smooth myocytes or in neural cells. They can present different shapes—globular, filamentous, and fusiform—and sizes, from a few microns to several centimeters. These and other physical features, such as the presence or absence of internal partitions and the ultrastructure of their walls, aid in species identification (Fayer 2004; Dubey 2015). A certain degree of size variation according to the age of the cyst and the type of parasitized host cell is sometimes observed. For example, within the same species, cysts in cardiac muscles are always smaller than those in skeletal muscles. The wall can invaginate forming villar protrusions, or cytophaneres, of different shapes and sizes. There are over 80 distinct types of cyst wall structures (Dubey 2015). Immediately underneath, there is a granular layer from which septa generally arise, separating the sarcocyst into compartments (Fig. 4.1a). Some sarcocysts, however, have no septa. Table 4.2 and

Table 4.2 Examples of sarcocysts and their special features

Species	Intermediate host	Sarcocyst				References
		Shape	Size (μm)	Type of wall	Distribution	
Sarcocystis tenella	Sheep	Round, elliptical, or elongated	≤700	Palisade-like, 1–3 μm thick	Worldwide	Dubey (2015) and Saito and Itagaki (1994)
S. arieticanis	Sheep	Oval	≤900	Hairlike of variable shape, thin (<1.0)	Worldwide	Dubey (2015)
S. neurona	Cat, skunk, raccoon, armadillo, sea otter, horse (aberrant)	Round	≤700	Striated	Worldwide	Dubey (2015) and Dubey et al. (2015)
S. aucheniae	South American camelids	Rice grain-like	≤5000	Cauliflower-like, 50 μm thick	South America	Carletti et al. (2013)
S. masoni	South American camelids	Oval	≤800	Cauliflower-like, 2.5–3.5 μm thick	South America	Moré et al. (2016)
S. cruzi	Cattle	Oval	≤420	Ribbonlike, thin (<1.0)	Worldwide	Dubey (2015) and Moré et al. (2010)
S. hominis	Cattle	Globular or oval	≤2,600	Fingerlike, thick (>6)	Worldwide	Dubey (2015)
S. gigantea	Sheep	Globular	≤10,000	Cauliflower-like, <2 μm thick		Saito and Itagaki (1994)
S. capracanis	Goat	Oval	≤1000	Honeycomb-like, 3 μm thick	Worldwide	Dubey (2015) and Saito and Itagaki (1994)

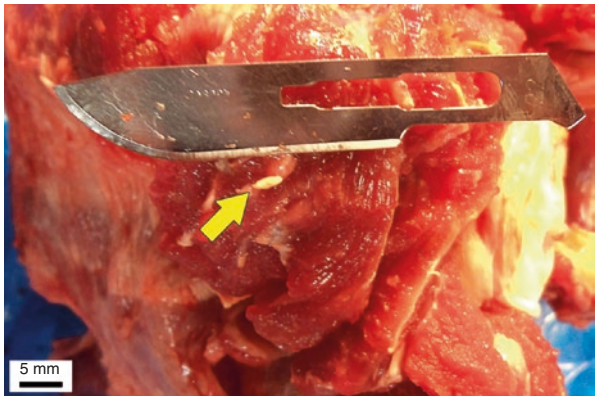

Fig. 4.2 Cyst of *Sarcocystis aucheniae* (*arrow*) found in muscle tissues of a llama (*Lama glama*)

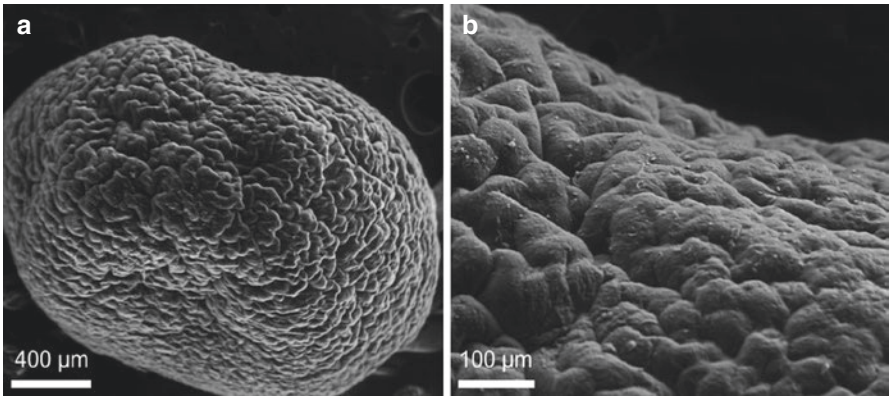

Fig. 4.3 Scanning electron microscopy of a *Sarcocystis aucheniae* cyst. View of the whole cyst showing a brain-like wall (**a**); detailed image of the cyst wall (**b**)

Figs. 4.2 and 4.3 show examples of sarcocysts and their special features. Parasites are located in the fluid contained between partitions (Fig. 4.1b) or, when no partitions are present, free within the cyst. The number of parasites contained in a sarcocyst varies with the species and the stage of maturation: young cysts as small as 5 µm in diameter might contain only two parasites, while a mature cyst can contain over 10^7 parasites, as is the case of *S. aucheniae* macrocysts (Carletti et al. 2013).

Metrocytes and bradyzoites are the parasite stages found in sarcocysts. Metrocytes—mother cells—are rapidly multiplying forms dominant in immature cysts. They are round to oval and have a variable size according to the stage of division. During the transformation of a merozoite into a metrocyte, many of the organelles of the apical complex, such as micronemes, conoid, and polar and apical rings, disappear, while ribosomes, endoplasmic reticulum, and mitochondria become more abundant, and the nucleus becomes larger. Bradyzoites, slow cells or also known as cystozoites, are the dominant forms in mature cysts. They are approximately 17 by 4 µm in size and display gliding motility and a characteristic apical complex (Figs. 4.4 and 4.5). In mature cysts, metrocytes localize in the cortex and

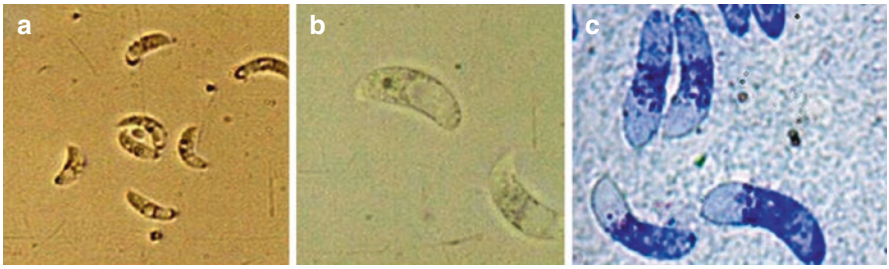

Fig. 4.4 Micrographs of *Sarcocystis aucheniae* bradyzoites. Phase-contrast microscopy (**a**), 400× and (**b**) 1000×, fixed drop stained with hematoxylin-eosin (**c**), 1000×; note the polarity of granulous material

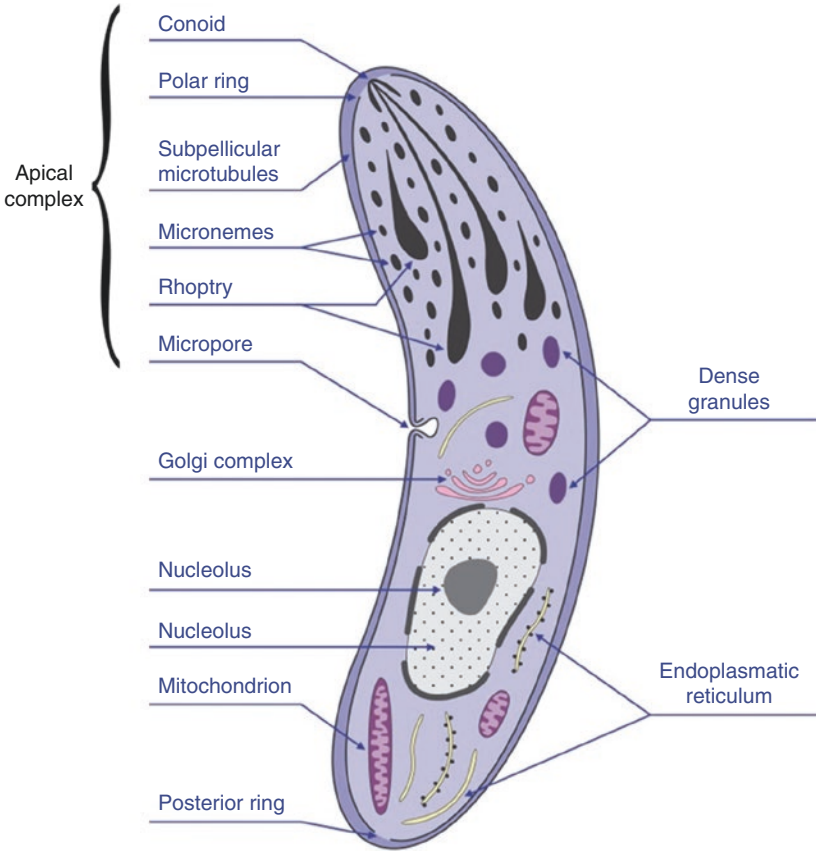

Fig. 4.5 Scheme of a *Sarcocystis* bradyzoite

stain lightly with hematoxylin and eosin, while bradyzoites are found in the medulla and get heavily stained (Dubey 2015).

Micro- and macrogamonts are formed upon bradyzoite infection of goblet cells of the small intestine of the definitive host. Macrogamonts are round or ovoid,

measure up to 20 μm in diameter, and contain a single large nucleus. Initially intracellular, they are usually freed into the lamina propria after lysis of the host cell. Microgamonts are elongated, slightly smaller than macrogamonts, and contain several nuclei that move to the periphery. Slender microgametes with two flagella, measuring up to 10 μm in diameter, are formed around each nucleus (Dubey 2015).

Oocysts result from the fusion of micro- and macrogametes. They have an ellipsoid shape, measure around 20 μm long, and are surrounded by a thin wall with a dense external layer and an internal layer of one to four membranes. When eliminated in the feces, they are sporulated and contain two sporocysts. Sporocysts measure 10 by 15 μm and are indistinguishable between species. Each sporocyst has four sporozoites arranged lengthwise (Fig. 4.6) (Dubey 2015; Fayer et al. 2015).

Sporozoites are banana-shaped cells measuring 11–19 μm long by 7–10 μm wide, with all the structural features of bradyzoites. In addition, they possess one or more virus-like crystalloid bodies that consist of electron-dense and electron-lucent granules. These structures likely represent a source of energy or amino acids, as was postulated for *Eimeria* sp. (Dubremetz and Torpier 1978).

4.1.2 Life Cycle

The biological cycle of *Sarcocystis* parasites remained unknown until 1972 when it was recognized that the predator-prey relationship corresponded to a definitive and an intermediate host, respectively. The life cycle consists largely of schizogony—also known as merogony—gametogony, and sporogony. The latter two comprise the sexual phase of the cycle and take place in the intestine of the definitive host—predator. Schizogony corresponds to the asexual phase and occurs in various tissues of the intermediate host—prey—until the formation of bradyzoite-containing cysts that are mainly located in muscle fibers, as mentioned before (Dubey et al. 1989) (Fig. 4.6).

A compatible herbivore becomes infected by ingesting pastures or water contaminated with sporulated *Sarcocystis* sporocysts. Exposure to trypsin and bile in the small intestine causes the liberation of four motile sporozoites from each sporocyst that invades endothelial cells of mesenteric lymph node arteries. Here, first-generation schizogony takes place, giving rise to numerous motile merozoites that bud from a schizont and are released to the bloodstream. Peripheral blood smears show the presence of merozoites between 24 and 46 days postinfection. Merozoites invade endothelial cells of downstream arterioles, capillaries, and veins, distributing throughout the body and producing additional generations by schizogony. Schizonts of some *Sarcocystis* species can also be found in connective tissue cells, macrophages, neural cells, and cells of many different organs. The last schizogony cycle takes place when a merozoite invades a muscle cell—skeletal, smooth, or cardiac—or, exceptionally, a nervous cell and forms a sarcocyst within its surrounding PV. Intracellularly, the invading merozoite first differentiates into a metrocyte, which reproduces by endodyogeny inside the PV. In this type of asexual reproduction, two daughter cells arise from an existing one, which is consumed during the

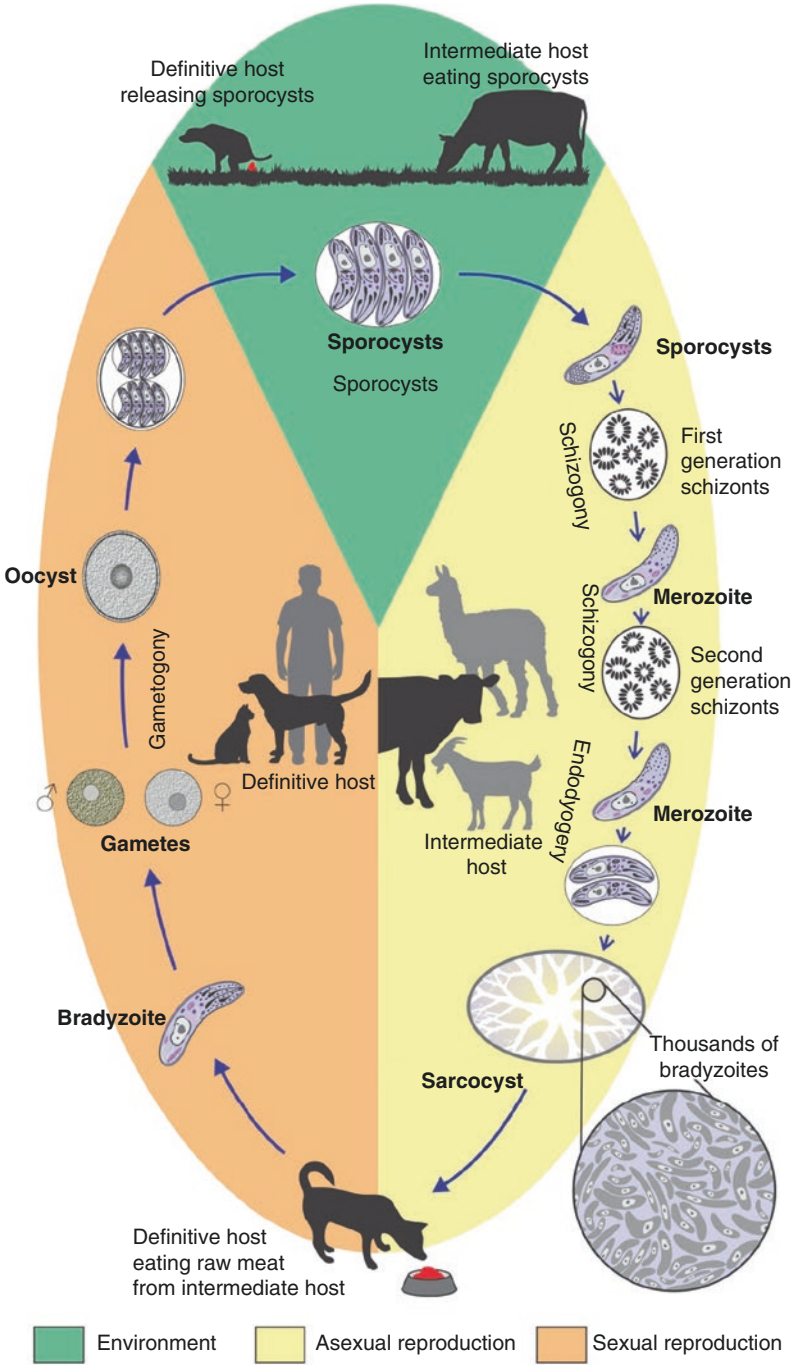

Fig. 4.6 Life cycle of *Sarcocystis*

Fig. 4.7 Dog feeding on raw meat during the slaughter of a llama (Catamarca, Argentina)

process. Concomitantly, a wall develops, isolating the nascent sarcocyst from the surrounding tissues. Eventually, metrocytes stop division and differentiate into infective bradyzoites, which display an apical complex that will aid in the invasion of definitive host cells. A sarcocyst full of bradyzoites is considered mature. The time elapsed between infection and formation of a mature sarcocyst varies between species but lasts in general around 2 months. Cysts can then persist in the tissues for months or even years. Bradyzoites are infectious for the definitive host, while schizonts and immature sarcocysts are not. The number and distribution of cysts in a particular host depend on different factors, including the amount of sporozoites ingested, the *Sarcocystis* and host species involved, the stage of infection, and the immune status of the animal. Gametogony is possible when a compatible carnivore feeds on cyst-containing tissues of an intermediate host (Fig. 4.7). Upon digestion of the cyst wall in the stomach and intestine, bradyzoites are liberated from the sarcocyst and penetrate host cells, generally goblet cells, or enterocytes of the small intestine. Each bradyzoite differentiates intracellularly into either a macro- or a macrogamont. Each macrogamont yields a single macrogamete, while microgamonts become multinucleated and yield several microgametes. The latter are motile and migrate to the surface of a macrogamete. After membrane fusion, the microgamete nucleus enters and fertilizes the macrogamete, yielding a zygote. A thin wall (<1 μm) then develops around the zygote giving place to the formation of the oocyst. The whole process of gametogony and fertilization can be completed in 1 day. The infected cells move to the lamina propria, where sporulation happens, giving rise to two elongated sporocysts containing four sporozoites each. The timing of sporocyst excretion by the definitive host after ingestion of sarcocysts is highly variable within the same species but in general starts after 7 and 14 days (Dubey et al. 1989; Dubey 2015; Fayer et al. 2015).

4.1.3 Host-Pathogen Interactions

Even though *Sarcocystis* is a very broad genus that includes some of the most prevalent parasites of vertebrate animals, they are understudied compared to other members of the Apicomplexa. For this reason, information on host-pathogen interactions is rather scarce. Since *Sarcocystis* sp. are obligate intracellular parasites, surface molecules that participate in recognition and invasion of host cells are likely essential for their survival. A prominent group of coccidian surface proteins are the SAGs, a family of glycosylphosphatidylinositol (GPI)-anchored surface antigens, initially characterized in *Toxoplasma gondii* and *Neospora* spp. (Howe et al. 2005). *T. gondii* SAGs have been implicated in receptor-ligand interactions with the host cell surface and in the stimulation of immune responses during infection, suggesting they are attractive targets for anti-coccidian drugs or immunotherapy approaches (Jacquet et al. 2001; Rachimel et al. 2004). Homology searches in an expressed sequence tag (EST) database of *S. neurona* allowed the identification of four SAG family members in this parasite, and other two members were later discovered (Howe et al. 2005; Crowdus et al. 2008). These proteins were demonstrated to be expressed on the surface of merozoites and to be highly immunogenic. The presence of SAG family proteins in different coccidian genera suggests a conserved essential function (Howe et al. 2005). Interestingly, studies performed with *S. neurona* merozoites, bradyzoites, and sporozoites showed that expression of individual SAGs is stage-specific. This is consistent with findings in *T. gondii* and suggests that surface antigen switching could be essential for the completion of the parasite life cycle (Gautam et al. 2011). Another GPI-anchored protein, surface protein 1 or SnSPR1, has been also identified in the *S. neurona* EST database. Contrary to SAGs, SnSPR1 shows no orthologs in other coccidian genera. It is expressed at the surface of merozoites in all stages of schizont development, is immunogenic, and might also participate in host-pathogen interactions (Zhang and Howe 2008).

Microneme proteins are also key elements in the invasion process of apicomplexan parasites, likely involved in the attachment and entry into the host cell (Dubremetz et al. 1998). The ortholog of a *T. gondii* microneme protein was identified in the *S. neurona* EST database and named SnMIC10. This protein has been shown to be differentially expressed in the apical end of merozoites during endopolygeny, supporting the view that micronemes are only needed during cell invasion (Hoane et al. 2003). A large array of conserved and species-specific proteins of *Sarcocystis* parasites await characterization and could serve as targets for control strategies.

Effector cells of the host immune system are mobilized during a *Sarcocystis* infection. The predominant cells infiltrating visceral and muscular tissues are lymphocytes and macrophages (Dubey et al. 1982). The cell infiltration of mononuclear cells starts during the third week of infection and can last for several months, even after the parasite is no longer detectable in visceral tissues (Gasbarre et al. 1984). Whether these cellular events participate in the recovery of the host from sarcocystosis has not been established, and passive transfer of resistance via cells or antibodies has not been reported. The intense cellular response seen in immune animals that

survive lethal challenges indicates a cell-mediated immunity against the parasite. Protective immunity has been shown to be induced only by homologous *Sarcocystis* species. As an example, in *S. hirsuta* experimentally infected cattle, no protection against challenge with *S. cruzi* was shown (Dubey 2015; Fayer and Dubey 1984; Ford 1985).

S. neurona-experimentally infected horses develop clinical disease and generate antibodies in serum and cerebrospinal fluid. Alterations in the immune cell subset expression that changed during disease progression were observed. Infected horses showed decreased antigen-specific proliferation responses compared to nonexperimentally infected horses, suggesting that the process between antigen-presenting cells—monocyte/dendritic cells—and/or T-cell antigen recognition may be damaged in *S. neurona*-infected horses (Lewis et al. 2014).

4.2 Diagnosis and Epidemiology

4.2.1 Diagnosis

Diagnosis of acute sarcocystosis is difficult since symptoms are not very specific and, therefore, easily confused with other pathological processes. Regularly, a diagnosis of sarcocystosis is based on the elimination of other causative agents, a good epidemiologic evaluation of the intermediate host, and its relationship to definitive hosts, as well as clinical findings (Cordero del Campillo et al. 1999). However, there are some techniques that have been employed or developed over the years and are described below.

4.2.1.1 Definitive Host

The diagnosis of sarcocystosis in the definitive host is based mostly on the identification of *Sarcocystis* sporocysts in the feces. This can be achieved by using a coproparasitological assay, consisting of flotation in zinc sulfate or other high-density solutions, followed by microscopic observation (Dubey et al. 2015). However, species cannot be discriminated by this method because of morphological similarities of sporocysts, thus molecular tests need to be employed.

4.2.1.2 Intermediate Host

Diagnosis can sometimes be inferred from epidemiological data of the region of interest, as well as information obtained by coproparasitological analysis of definitive hosts. Usually, confirmatory diagnosis is achieved by postmortem examination of the skeletal muscle. Cysts of some species are visible to the naked eye, as in the case of *S. aucheniae* and *S. gigantea*, that infect South American camelids and sheep, respectively (Fig. 4.2). In a great number of species, however, cysts are microscopic, so other examination methods are applied.

Microscopy and electron microscopy allow to diagnose *Sarcocystis* sp. and to differentiate morphological features between species. They are specific but time-consuming, limiting their application on large numbers of samples (Moré et al. 2010).

Artificial digestion is a sensitive method that allows analyzing large amounts of tissue. It detects bradyzoites released from cysts, but it does not allow to differentiate between species of *Sarcocystis* (Savini et al. 1996). In the digestion procedure, tissues are incubated with proteases, such as trypsin or pepsin. Released bradyzoites can be used as antigen, and the species can be identified by molecular methods.

Histopathology and examination of fresh tissue allows the differentiation between thick- and thin-walled cysts, but not between species. The sensitivity of histopathological examinations is lower due to the smaller volume of sample that can be processed (Dubey et al. 1989).

Polymerase chain reaction (PCR) is an important tool for epidemiological studies. It allows to detect DNA of the parasite in small volumes of sample and also to differentiate between *Sarcocystis* and related organisms, such as *Toxoplasma* and *Neospora,* or discriminate between *Sarcocystis* species (Ortega-Mora et al. 2007). Different PCR protocols have been developed over the years. For example, recent studies showed that it is possible to detect DNA of *S. aucheniae* in the blood of South American camelids employing a semi-nested PCR (Martín et al. 2016). Other studies allowed the differentiation of *Sarcocystis* species affecting cattle using multiplex real-time PCR (Moré et al. 2013) or applied PCR followed by RFLP—*restriction fragment length polymorphism*—to determine the *Sarcocystis* species affecting sheep (Hamidinejat et al. 2014). In addition to PCR, another highly sensitive DNA amplification technique that takes place at constant temperature, and thus does not require the use of a thermocycler, is being increasingly applied to the diagnosis of different pathogens (Notomi et al. 2015). This technique—known as *loop-mediated isothermal amplification* or LAMP—has been successfully applied to the molecular detection of *S. fayeri* in horsemeat (Furukawa et al. 2016).

In immunohistochemistry, anatomical, immunological, and biochemical techniques are combined to identify discrete tissue components. The method is based on the interaction of target antigens with specific antibodies tagged with a label that allows visualizing the distribution and localization of specific components within cells and in the proper tissue context. A number of immunohistochemical methods have been developed for the improvement of the sensitivity and specificity of the histological detection of life cycle stages of *Sarcocystis* species. However, these methods are complicated due to the high cross-reactivity among *Sarcocystis* species when polyclonal antisera are used. In addition, cross-reactivity of anti-*Sarcocystis* sp. antibodies is sometimes extended to other cyst-forming coccidia such as *T. gondii* (Uggla and Buxton 1990). Monoclonal antibodies have been produced for *S. cruzi, S. tenella,* and *S. arieticanis*; however also in this case, most are cross-reactive with antigens of heterologous *Sarcocystis* species (Ortega-Mora et al. 2007).

Enzyme-linked immunosorbent assay (ELISA) detects and measures antibodies and is the most commonly used serological test for the diagnosis of *Sarcocystis*. However, cross-reactivity with heterologous *Sarcocystis* species is also a problem here (Tenter 1995). Different types of antigens have been employed in ELISA tests. As an example, an indirect ELISA (iELISA) based on *S. cruzi* bradyzoite antigens was developed in Sri Lanka for the detection of anti-*Sarcocystis* spp. antibodies in

cattle (Kalubowila et al. 2004). In addition, in Argentina, an iELISA based on an immunogenic protein fraction extracted from *S. aucheniae* bradyzoites was applied to the detection of anti-*Sarcocystis* antibodies in South American camelids (Romero et al. 2014). More recently, in the USA, recombinant forms of *S. neurona* SnSAG surface antigens were used in an iELISA format to measure antibodies in serum and cerebrospinal fluid. The latter revealed active infection in the central nervous system (Yeargan et al. 2015).

In a Western blot, a mixture of proteins is separated by gel electrophoresis and then transferred to a membrane. The membrane is incubated with specific antibodies against the protein or proteins of interest. Detection is achieved by reaction with an enzyme-conjugated anti-species antibody, followed by incubation with a colorimetric or chemiluminescent substrate (Mahmood and Yang 2012). Detection of antibodies against two *S. neurona*-specific antigens, of 29 and 17 kDa, is currently the standard serological diagnostic method for infections with this parasite in horses (Rossano et al. 2000; Hamir and Dubey 2001).

4.2.2 Epidemiology

Sarcocystis infections of farm animals are worldwide distributed and often present high prevalences, both in developing and industrialized countries (Dubey et al. 1989). The percentages of infected animals depend on various aspects, including host, viability of the sporocysts in the environment, number of sporocysts released by the definitive host, immune status of the intermediate host, hygiene, and proximity between definitive host and intermediate hosts, among others (McKenna and Charleston 1994; Savini et al. 1996). According to the species, infections can cause mortality, morbidity, abortions, lower meat yield, and economic losses due to confiscation of meat when macroscopic cysts are found (Poulsen and Stensvold 2014). In cattle, *Sarcocystis* infections are regularly asymptomatic, with prevalences over 90%, being *S. cruzi* the most commonly found species (Moré et al. 2010). In Argentina, recent studies showed a direct connection between the type of breeding of llamas and prevalence of anti-*Sarcocystis* sp. antibodies (Romero et al. 2017). In Bolivia, a study made in abattoirs showed that 23–50% of llama carcasses contained macroscopic cysts, with infection rates higher in females and in older animals (Rooney et al. 2013).

Seroprevalences reported for *S. neurona* in US horses vary from 15 to 89%, depending on the geographic region. They are also lower during the winter season compared to the rest of the year (Pusterla et al. 2014; Reed et al. 2016). Seroprevalences of around 35% were reported in Brazil and Argentina, indicating that the parasite is also present in South America (Dubey et al. 1999a, b). Most infections have been observed in young animals of 1–5 years or older than 13 years of age. Interestingly, the likelihood of infection was significantly reduced in farms where wildlife had no access to feed and when a creek or river was present as a water source. On the other hand, stress related to heavy exercise, transport, injury, surgery, or parturition was found to increase the risk of disease caused by *S.*

neurona. In addition, racehorses and show horses had higher infection risks than breeding and pleasure horses (Reed et al. 2016).

Sarcocystosis is usually acquired horizontally through ingestion of contaminated food or water. In addition, anti-*Sarcocystis* sp. antibodies were detected in horse fetuses and newborn foals, indicating the occurrence of transplacental transmission. However, this event appears to be rare (Duarte et al. 2004).

A number of molecular typing techniques, including PCR-RFLP, microsatellite, and whole-genome fingerprinting, have been developed to differentiate between *S. neurona* isolates. Application of these techniques can aid in understanding parasite transmission and epidemiology and reveal its population structure (Elsheikha and Mansfield 2007).

4.3 Clinical Effects, Prevention, and Treatment

4.3.1 Clinical Effects

Sarcocystis natural infections of intermediate hosts are in most cases asymptomatic. However, *S. neurona* is a special case, due to the parasite tropism for horse nervous tissues, infecting both gray and white matter, which provokes focal or multifocal signs of neurological disease. This syndrome was initially known as segmental myelitis and, later, focal encephalitis-myelitis, until the presence of protozoa in characteristic lesions led to the current name of equine protozoal myeloencephalitis (EPM). The disease can actually also be caused by *Neospora hughesi*, although the majority of cases are due to *S. neurona*. Clinical EPM signs include dysphagia, upper airway dysfunction, muscle weakness and atrophy, ataxia, weakness of limbs, and even seizures. Severely affected horses show difficulty in standing, walking, or swallowing and present head tilt and facial nerve paralysis. In some cases, signs stabilize but relapse in a few days or weeks (Dubey et al. 2015; Reed et al. 2016).

Experimental infections of different farm animals and pets, on the other hand, have shown a number of severe signs (Table 4.3). As a rule in *Sarcocystis* infections, necrosis of cells and tissues produced by multiplication of schizonts is very common, but it does not appear to be extensive enough to cause severe illness or death in large animals—cattle, sheep, goats, and pigs. However, an intense inflammatory reaction is usually associated to second-generation schizont maturation. Eosinophilic myositis (EM) is a specific inflammatory condition of striated muscles. It happens during the penetration of myocytes by merozoites and might be related to products liberated from merozoites or myocytes. It has been found mostly in cattle, occasionally in sheep, and rarely in pigs and horses. With the progression of EM, eosinophils and myocytes degenerate, resulting in granulomas with a central area of necrosis. Later, the tissue becomes surrounded by zones of giant cells, epithelial cells, lymphocytes, and fibrocytes (Dubey 2015).

Abortion can result when animals become infected with pathogenic species of *Sarcocystis* during pregnancy, and there are many unanswered questions and observations that appear contradictory about the effect of sarcocystosis on fetal health.

Table 4.3 Clinical effects of *Sarcocystis* spp. infections of farm animals and pets

Intermediate host	*Sarcocystis* species	Clinical effects (depending on the number of sporocysts ingested)
Pig (*Sus scrofa*)	*S. miescheriana*	Weight loss, purpura of the skin, dyspnea, muscle tremors, diarrhea, myositis, abortion, and death
	S. suihominis	
	S. porcifelis	
Cattle (*Bos taurus*)	*S. cruzi*	Fever, weight loss, anemia, hair loss, weakness, prostration, abortion, reduced milk yield, hypersalivation, neurologic signs, and death
	S. hirsuta	Fever, diarrhea, and anemia
	S. hominis	Anemia
Sheep (*Ovis aries*)	*S. tenella*	Anorexia, weight loss, fever, anemia, loss of wool, abortion, premature birth, neural signs, myositis, and death
	S. arieticanis	Fever, less wool growth, and death
	S. gigantea	
Goat (*Capra hircus*)	*S. capracanis*	Fever, weakness, anorexia, weight loss, tremors, irritability, abortion, and death
Equine (*Equus* spp.)	*S. fayeri*	Mild anemia, hair loss, and fever
	S. equicanis	Fever, apathy, and anorexia
	S. neurona	Ataxia, general weakness with muscle spasticity, loss of appetite, decreased tongue tone, and facial paresis
South American camelids (*Lama* spp.)	*S. aucheniae*	Necrosis, hemorrhage, eosinophilic myositis, abortion, and death
Dog (*Canis familiaris*)	*S. caninum*	Fever, apathy, anorexia, muscle weakness, ataxia, and elevated liver and muscle enzymes
Chicken (*Gallus gallus*)	*S. wenzeli*	Myositis, muscular weakness, and neurologic signs
	S. horvathi	

With the exception of *S. neurona*, where signs correspond to natural or experimental infections, all other effects were registered exclusively in experimental infection studies (Dubey 2015; Fayer et al. 2015)

When infection is induced experimentally, most animals can develop clinical sarcocystosis and abortion, showing parasites and lesions in maternal placentomes but rarely infecting the fetus or fetal membranes. Unlike experimentally infected animals, those with natural infections show parasites, lesions, or both in the fetuses (Jerrett et al. 1984).

Definitive hosts usually do not present clinical signs. Dogs, cats, coyotes, foxes, and raccoons fed with tissues infected with different *Sarcocystis* species-excreted sporocysts but were otherwise asymptomatic. However, a few dogs and coyotes vomited or were anorexic for 1–2 days following ingestion of meat. In addition, in trials made in human volunteers who ingested beef and pork infected with *S. hominis* or *S. suihominis*, respectively, clinical symptoms were observed, including vomiting, diarrhea, and respiratory distress (Dubey 2015). Indeed, soluble extracts prepared from the tissue cysts of various *Sarcocystis* species have been shown to

contain powerful toxins—sarcotoxins—which have even proven lethal when administered to laboratory animals (Hiepe et al. 1981; Harada et al. 2013, Kamata et al. 2014). Accordingly, experimental inoculation of rabbits with an extract derived from *S. fusiformis* or *S. cruzi* cysts developed a shock-like state and/or death, likely caused by the sudden exposure to high doses of sarcotoxins (Saleque et al. 1991; Nakamura et al. 1999).

4.3.2 Prevention

Currently, there is no vaccine to protect animals against sarcocystosis, but experimental studies indicate that cattle, sheep, goats, and pigs develop a humoral response when inoculated with small numbers of live sporocysts. For this reason, there is hope of developing a vaccine for sarcocystosis in the future (Dubey 2015). In the USA, a killed whole *S. neurona* merozoite vaccine was marketed by Fort Dodge, but the product has been retired from the market, because no differences between vaccinated and control horses were found during the trials (Dubey et al. 2015).

For the time being, interrupting the cycle of the parasite is the only practical method of control. This can be achieved by preventing definitive hosts to consume raw or insufficiently cooked meat infected with *Sarcocystis* or to cohabitate with intermediate hosts.

It is important to take into account that during veterinary inspections in slaughterhouses, meat containing macroscopic cysts can be confiscated, but microscopic cysts pass unnoticed. For this reason, it can be assumed that most meat that is consumed is infected with *Sarcocystis* (Godoy et al. 2007). It has been demonstrated that freezing meat is effective to prevent the occurrence of food poisoning when consuming raw meat containing sarcocysts. Indeed, freezing *S. fayeri* sarcocyst-infected horsemeat for 48 h at −20 °C resulted in the disappearance of bradyzoites, as well as of a 15 kDa sarcotoxin found to be responsible for the clinical signs associated with food poisoning (Kamata et al. 2014).

Confiscated carcasses should be buried or incinerated to prevent definitive hosts from eating infected meat, and the prophylactic use of anticoccidials in definitive and intermediate hosts could help to control sarcocystosis in farm animals and pets (Dubey 2015).

4.3.3 Treatment

Currently there is no specific prophylactic or therapeutic treatment for sarcocystosis. Infections usually go undetected, but in case of the appearance of clinical signs, some drugs have proved effective to partially reduce illness and parasitic load (Table 4.4). In horses, treatment with an anticoccidial drug has been shown to increase ten times the likelihood of recovery from clinical EPM signs and survival. Treatment, however, was more effective when milder, rather than severe, clinical signs were present (Saville et al. 2000; Pusterla et al. 2014; Reed et al. 2016).

Table 4.4 Drugs used for the treatment of sarcocystosis (Cordero del Campillo et al. 1999; Dubey 2015; Fayer 2004; Lindsay and Dubey 1999; Reed et al. 2016)

Drug	Target host	Action
Amprolium	Cattle, sheep, goat, pig, chicken, and dog	Coccidiostat Blocks the thiamine transporter
Decoquinate	Poultry, cattle, sheep, goat, and equine	Coccidiostat
Diclazuril	Poultry, cattle, sheep, goat, and equine	Coccidiostat
Dihydrofolate reductase inhibitors	Equine	Inhibits folate's actions
Salinomycin	Animals	Coccidiostat
Halofuginone	Animals	Coccidiostat. Inhibits T-helper 17 cells
Lasalocid	Cattle, sheep, and poultry	Coccidiostat
Ponazuril	Equine	Coccidiostat. Inhibits mitochondrial function
Primaquina	Definitive hosts	Gametocide. Inhibits the synthesis of polyamines
Robenidine	Sheep and goat	Coccidiostat
Sulfadoxine and pyrimethamine	Definitive hosts, equine	Inhibits dihydropteroate synthetase activity
Sulfadoxine and trimethoprim	Cattle, sheep, goat, and pig	Inhibits dihydropteroate synthase

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