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Review

SARCOCYSTIS SPP., A PARASITE WITH ZOONOTIC POTENTIAL

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Summary

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Sarcocystosis infection is caused by protozoan cysts of genus *Sarcocystis* spp. where *S. hominis, S. heydorni* (bovines) and *S. suihominis* (porcine) are the most relevant for humans because of their zoonotic potential. *S. cruzi, S. suihominis* and *S. ovicanis* represent the most pathogenic species for cattle, pigs and sheep respectively. This infection has a worldwide importance due to its high transmission; besides to represent a zoonosis, it generates great economics losses. The main diagnostic methods for this disease are artificial digestion, PCR, indirect ELISA, and compression analysis. It's important to highlight few studies on *Sarcocystis* spp., especially the ones involving the pursuit of effective treatments to control the infection for both humans and animals, however, some studies have reported that treatments such as cotrimoxazole and albendazole with or without prednisone are effective in counteracting symptoms in humans, considering the lack of reports about *Sarcocystis* spp. prevalence in Colombia.

Key words: economic losses, prevalence, symptomatology, *Sarcocystis* spp., zoonosis

INTRODUCTION

Sarcocystis spp. is a protozoan, intracellular parasite of the phylum Apicomplexa, widely distributed in the animal kingdom, reported by Miescher in 1843 who observed long, thin and white cysts in the muscles of a domestic mouse in Switzerland, without a scientific name (Dubey *et al.*, 1989). However, this parasitic genus was first described by Lankester in 1882 after his experimentation in rats (Fayer, 2004).

This parasite has an indirect life cycle, it is transmitted from its intermediate host or prey that can be an herbivore or an om-

nivore, to its definitive host or predator that is usually a carnivore (Lucas, 2012; Pulido *et al.*, 2013). It is assumed that *Sarcocystis* species have a specific intermediate host, but an herbivore can serve as an intermediate host for several species of *Sarcocystis* (Dahlgren & Gjerde, 2007). The definitive host gets infected by ingesting cysts of the parasite found in the muscle tissue, this host can be a dog, a cat, or a human (Anonymous, 2005).

Sarcocystis has a low pathogenicity for the definitive host and it rarely causes diseases. However, some species are zoonotic (Lobão *et al.*, 2017); in the case of humans, they can act as a definitive host for two species of *Sarcocystis.* Their intermediate hosts are cattle, which carry *Sarcocystis hominis* and pigs which carry *Sarcocystis suihominis* (Dubey, 2015). Some highly pathogenic species are reported in the intermediate host; in the case of cattle these are *S. cruzi, S. hirsuta, S. hominis, S. rommeli* and *S. heydorni*, in pigs *S. suihominis* and *S. miescheriana*; in ovines *S. ovicanis, S. medusiformis*; in goats *S. capracanis, S. hircicanis*, and *S. moulei*, and in camels *S. cameli and S. ippeni* (Inga, 2014; Meistro *et al.*, 2015; Amairia *et al.*, 2016; Hu *et al.*, 2016; Dubey *et al.*, 2017; Yang *et al.*, 2018).

This parasite has great impact on public health, for it can be easily worldwide spread. This foodborne disease can be found on raw or undercooked meat, which makes it a zoonotic parasite that causes different symptoms in humans. Correspondingly, the presence of this parasite in livestock production has a very important role at an economic level, since it generates losses in the carcasses of cattle, pigs and sheep, a decrease in milk production in both sheep and cattle, and low wool quality in sheep (Poulsen & Stensvold, 2014). For this reason, the objective of this article is to carry out an updated bibliographic review of *Sarcocystis* spp., considering its life cycle, pathogenesis, symptomatology, treatment, among other important factors.

BIOLOGICAL CYCLE OF *SARCOCYSTIS* SPP.

Sarcocystis spp. species develop in a two-host life cycle that includes an intermediate and a definitive host (Fraser, 1993; Lucas, 2012). Humans can serve as an intermediary and definitive host for different *Sarcocystis* species (Lindsay & Weiss, 2004). Consequently, humans are definitive hosts when they consume tissue cysts with pork or undercooked beef (sarcocysts) and in nature, carnivores acquire infection by consuming prey with tissues harbouring cystic parasites (Rosenthal, 2015).

The life cycle of *Sarcocystis* constitutes of merogonial, gamogonial and sporogonial phases (Rassouli *et al.*, 2014). The asexual stage of *Sarcocystis* (merogonia) only develops in the intermediate host after the ingestion the sporocysts with food or contaminated water by faeces. Then the sporozoites are released and begin their development in the form of schizont in the vascular endothelium, the sporozoites penetrate the intestinal epithelium and invade the endothelial cells in the arteries of the mesenteric lymph nodes where they undergo an asexual multiplication (called schizogony or merogony) producing numerous merozoites, released by mature schizonts and leading to the second generation of endothelial schizonts. Merozoites originating in this second generation invade skeletal and cardiac myocytes as well as neurons, and become metrocysts that go through a series of internal mitotic divisions. When it

is full of bradyzoites, the metrocyst becomes a sarcocyst that will eventually be infectious when consumed by the definitive host (Fraser, 1993; Rassouli *et al.*, 2014; Rosenthal, 2015).

Subsequently, the sexual stage (gamogony) is carried out in the definitive host, and the organism is spread through the ingestion of muscle with sarcocysts (infected meat), these contain hundreds of bradyzoites inside them, which one day after ingestion of infected meat become male and female gamone in the small intestine. It is important to clarify that in the definitive host there is no multiplication of the parasite; therefore, gametogony generally occurs in the lamina propria of the small intestine and leads to the formation of non-sporulated oocysts, the oocysts sporulate *in situ*, usually within a week after ingestion of bradyzoites. Later, the host begins to expel infectious sporocysts in the feaces after one week (Dubey, 2015). The wall of these sporocysts is delicate and often breaks, releasing individual sporocysts with four sporozoites (Lindsay & Weiss, 2004; Ortega-Mora *et al.,* 2007; Dubey, 2015).

PATHOGENY

The pathogenicity and the biological cycle were determined through various investigations, which allowed demonstrating that asexual reproduction of the parasite is the cause of the different vascular endothelial lesions of the capillaries and arterioles of most organs of the animal (Jauregui, 2017). There are certain *Sarcocystis* species that have a high degree of pathogenicity for their intermediate hosts, including *Sarcocystis cruzi, S. suihominis* and *S. ovicanis* which, even in mild infections, can cause death (Inga, 2014).

The pathogenicity of *Sarcocystis* species depends on different factors such as the ability to multiply, the location of schizogony, the proliferation of schizonts and the possibility of reaching the central nervous system (Jauregui, 2017). On the other hand, it has been considered that the infective dose and the chronological rhythm of the reinfections in relation to the *Sarcocystis* species play a very important role in the pathogenesis. Regarding the predisposing factors in the host, the immunological state is considered; therefore, gestation, stress, nutritional status and lactation tend to favour the severity of the infection (Galvis & Agudelo, 2010).

The biological cycle of the parasite carried out inside the host can explain the different symptomatology. It has been determined that the oocysts vary in size according to the species, they are of the *Isospora* type (2 sporocysts with 4 sporozoites each) and they are already sporulated. In the definitive host, the wall of the oocyst is very thin and often breaks down leaving sporozoites in the intestine free, which penetrate into the epithelial cells of the small intestine where gametes develop immediately. The engendered zygotes evolve into mature oocysts, those that leave the intestine (Baldeón, 2012); this process of parasitic reproduction produces the secretion or excretion of substances resulting from *Sarcocystis* metabolism which induces the release of mediators of inflammation (Jauregui, 2017). Enteritis with infiltration of polymorphonuclear cells (PMN) and presence of eosinophils occurs. Necrosis is observed as possible sequel of an autoimmune reaction (Becerril, 2011).

The development in the intermediate host begins with the proliferative phase. It is pointed out that when the host cells break as a result of the asexual multiplica-

tion of the parasite in the endothelial cells of the intimal vascular tunica, it causes endarteritis and increase of capillary permeability, leading to extravasation of fluids, blood and mobile cells. An increase in systemic pressure may occur as a result of the obstruction of the vascular lumen caused by cellular debris that persisted in the walls of the vessels and in the bloodstream, this is demonstrated by the appearance of oedema and haemorrhages (Jauregui, 2017).

If the infection occurs in pregnant females, the asexual multiplication takes place in placental cotyledons, myoepithelial cells and occasionally, in foetal adnexa; leading to the appearance of extensive areas of mononuclear infiltration and tissue necrosis (Cordero *et al.*, 1999). This leads to the presence of abortions and foetal deaths.

The second cystic phase is characterised by two types of injuries; the first type is eosinophilic myositis, frequently described in cattle, related to the existence of high levels of IgE and high intensity of muscle parasitisation. The second type is the most frequent and constant, characterised by infiltration of mononuclear cells in the perivascular zone and in the periphery of apparently healthy parasitic muscle fibres (Jauregui, 2017).

Immature sarcocysts in the muscle can be detected 45 to 60 days after the ingestion of sporocysts, and are infective at approximately 70 days (Romero, 2009). After the formation of cysts, a pathological response has not been determined but the intermediate host presents difficulty in its growth (Godoy, 2006).

INMUNE RESPONSE

The immune response in the intermediate host against the protozoa is similar to the one triggered against bacteria (Inga, 2014). Where the protective antigens are associated with the formation of the sexual phases, the expression of the immunity depends on the activity of the T cells which collaborate with the production of neutralising antibodies against the sporozoites besides the release of lymphokines that try to inhibit the multiplication of the intracellular phases; to achieve reduction of clinical symptoms and reduction of the number of oocysts (Urquhart *et al.*, 2001).

As could be supposed for an intracellular parasite, the cellular immunity is mobilised during infection by *Sarcocystis*. Cellular infiltration has been observed in affected visceral and muscle tissues, mostly by macrophages and lymphocytes. This infiltration can remain for a long time. On the other hand, the existence of lymphocytes specifically to *Sarcocystis* in the peripheral circulation of bovines has been demonstrated within 15 days after infection, but their activity decreases rapidly, unlike in sheep, in which lymphocytic activity was evidenced between the third and fourth week (Inga, 2014).

Regarding the humoral response, some animals have been inoculated with *Sarcocystis*. In cattle (*S. cruzi*), IgM was developed at the third and fourth week post infection and returned to pre-infection levels after 2 to 3 months; the IgG1 response increased on the fifth and sixth post infection weeks, remaining high for more than 5 to 6 months (Romero, 2009). In ovines (*S. ovicanis*) there was no IgM, on the contrary, IgG antibodies had a similar course to what was observed in cattle (Inga, 2014). In pigs, an increase in IgM was identified from 21 days post infection, followed by a gradual decrease in terms of IgG positive values from 34 post infection day, which remains at least 160 days (Romero, 2009).

ZOONOSIS

The presence of *Sarcocystis* has been demonstrated in numerous animal species (mammals, birds and poikilothermic hosts), but only cattle and pigs are confirmed as intermediate hosts of the zoonotic *Sarcocystis* species (Dubey *et al.*, 2015a). Of the five species of *Sarcocystis* in cattle (*S. cruzi, S. hirsuta, S. hominis, S. rommeli* and *S. heydorni*), *S. cruzi* is the most important, it is the most frequent and the most pathogenic in cattle (Amairia *et al.*, 2016), but only *S. hominis* and *S. heydorni* are zoonotic (Dubey, 2015; Dubey *et al.*, 2015b; 2016). For pigs, there are three species of *Sarcocystis*, *S. miescheriana, S. suihominis* and *S. porcifelis,* only *S. suihominis* is zoonotic (Dubey *et al.*, 2015a). However, approximately 100 cases have been reported, where humans have proved to be intermediary hosts of some species that have not yet been identified, it is suspected that one of these may be *Sarcocystis nesbitti,* in which the snake fulfils the role of definitive host. This species of *Sarcocystis* is the one that has been most commonly reported as the cause of muscular sarcocystosis in humans (Lau *et al.*, 2013, 2014; Esposito *et al.*, 2014; Italiano *et al.*, 2014). It is believed that the mechanism of transmission occurs from the ingestion of food and water contaminated with the faeces of an infected predator (Falcón *et al.*, 2010).

CURRENT SITUATION IN THE WORLD

This parasite is widely distributed worldwide, especially in the Americas and Europe. Some studies report that *S. hominis* has not been detected in the United States, but a general rate of 15% is reported; while it has been reported that up to 63% of livestock in Germany is infected. *S. suihominis* was found more prevalent in Germany than in Austria. In Euro-Asian countries such as Russia, China and Japan, a prevalence of 10% is reported, while in Spain it varies between 35 and 100% (Pena *et al.*, 2001; Pulido *et al.*, 2013).

On the other hand, in Brazil, the 50 samples of raw kibbe from 25 Arab restaurants in Sao Paulo contained sarcocysts, among which 94% belonged to *S. hominis*, 70% to *S. hirsuta* and 92% to *S. cruzi* (Pena *et al.*, 2001). The overall prevalence of *Sarcocystis* in pigs worldwide is low, with a percentage from 3% to 36%. *S. suihominis* and *S. hominis* have been reported in slaughtered pigs and cattle reared in Japan (Lindsay & Weiss, 2004). In Colombia, this disease hasn't been investigated enough but the presence of *Sarcocystis* spp. in cattle for slaughter and in livestock production is reported (Zaldivar, 2007; Andrade *et al.*, 2009).

According to some studies, it was determined that intestinal sarcocystosis in humans is more prevalent in Europe than in any other continent (Dubey *et al.*, 1989). Additionally, it is established that muscular sarcocystosis in humans is less frequent, therefore there are not many reports of this worldwide, but even so, cases have been reported mainly from tropical and subtropical regions of Asia, the United States, Central America and from the South (Clavel *et al.*, 2001).

SYMPTOMATOLOGY

Symptomatology in the definitive host

Regarding symptomatology in humans with muscular sarcocystosis, febrile myositis has been mainly reported, as well as

severe myalgia, fever, fatigue, vasculitis, bronchospasm, pruritic eruptions, headache and arthralgia (AbuBakar *et al.*, 2013; Esposito *et al.*, 2014; Slesak *et al.*, 2014; Tappe *et al.*, 2014; Günther *et al.*, 2015).

In intestinal sarcocystosis, there is more information provided by different authors with reference to the symptoms that patients may present, indicating that in most of the cases the person infected with this type of sarcocystosis shows no obvious signs or symptoms, however, subclinical symptoms could present with transitory and mild gastrointestinal signs, like nausea, vomiting, acute or severe enteritis, or chronic enteritis. The differences depend on the number, and perhaps the species of the ingested *Sarcocystis*. There are few accurate data available on the duration of infection or the number of oocysts and sporocysts excreted (Schnieder, 2003; Fayer *et al.*, 2015), although it has been identified that in symptomatic patients, signs are evident two weeks post infection (Slesak *et al.*, 2014). Some cases have been reported in which humans with sarcocystosis have presented vasculitis, but no direct relationship with the presence of the parasite has been established (Lindsay & Weiss, 2004).

Symptomatology in the intermediate host

In the acute form, a variety of clinical signs are described, including fever, hyperthermia, apathy, depression, inappetence, polypnea and tachycardia. In the chronic form, the animals lose weight, in calves and lambs it happens after three weeks; in adults since the eighth post infection week. Also, mucous-cutaneous signs have been described, alopecia in the distal portion of the four limbs, ears and in the tail base in cattle. In sheep, the wool becomes brittle (Lindsay & Weiss, 2004). Interdigital injuries, laminitis, erosions in carpus and tarsus were reported in calves.

The mucous membranes are pale and by palpation, generalised peripheral lymphadenitis is detected. Nervous signs have also been described in sheep and goats including alteration of behaviour, stupor, ataxia, opisthotonos, nystagmus and galloping gait. Other signs have been reported, including exophthalmia, sialorrhea, submandibular oedema, haematuria, and urination disorders. Abortions can occur in gestating females, mostly in sheep and goats (Poulsen & Stensvold, 2014; Jauregui, 2017). Anaemia is detected after the third or fourth week, usually normocytic normochromic, however, cases of macrocytic/hypochromic anaemia have been reported in calves (Jauregui, 2017).

In severe *S. cruzi* infections, fever, anorexia, cachexia, decrease in milk production, diarrhea, muscle spasms, anemia, hyperexcitability, weakness, prostration and death occur; infections in gestating females in the last trimester of pregnancy could finish with abortion. Calves recovering from severe disease may have poor development and eventually die from cachexia. Infections are more important in ruminants and growing pigs, since they cause subclinical anaemia and decreased weight gain (Taylor *et al.*, 2007).

In pigs, the infection is very common but generally asymptomatic, however, simultaneous high consumption of parasites can cause severe disease in the second week of infection, presenting similar symptoms to those in ruminants such as fever, apathy, dyspnea, anaemia, hyperexcitability, muscular spasms, prostration and abortions (Baldeón, 2012).

POST MORTEM FINDINGS

In cattle, the tongue, heart, esophagus, diaphragm and latissimus dorsi muscle

should be analyszed for post mortem findings, considering that in these muscular tissues more microcysts in the fresh examination can be found, this can also be determined by histological examination, which is more accurate for the identification (Jauregui, 2017).

In a study carried out by Jauregui (2017), the prevalence of sarcocystosis was determined by the anatomical location in fresh sample and histopathological examination, detecting 59.05% and 71.43% in the tongue; 84.54% and 77.78% in the heart, 95.71% and 96.19% in the oesophagus; 61.43% and 67.14% in the diaphragm 82.38% and 64.29% in latissimus dorsi respectively. In another study, a percentage of 100% was found in the heart, followed by 71% of *Sarcocystis* found in esophagus and 28% in the diaphragm (Moré *et al.*, 2008).

Similarly, pigs' muscles should be also inspected, observing macroscopically the presence of cysts (Baldeón, 2012). At the histological level, multifocal degeneration and necrosis of the myocardial fibres can be found with interstitial oedema, severe nonsuppurative multifocal myocarditis, hepatitis, and non-suppurative interstitial nephritis (Caspari *et al.*, 2011).

DIAGNOSTIC METHODS

For diagnosis of *Sarcocystis* spp., one of the main methods is artificial digestion. This technique brings greater sensitivity compared to other methods used, allowing the detection of the bradyzoites released from *Sarcocystis*, but not identification of the species (Borji & Parandeh, 2010; Moré *et al.*, 2011). Histopathological examinations do allow the differentiation between thick cysts (≥3 µm) and thin-walled cysts (<1 µm), but no species differentiation within thick-walled cysts (*S. hirsuta* and *S. hominis*) (Moré *et al.*, 2011). However, the sensitivity of the histopathological examination is lower, due to the smaller volume of sample that can be processed (Dubey *et al.*, 1989).

On the other hand, the polymerase chain reaction (PCR) can be used to perform the molecular diagnosis of *Sarcocystis,* which represents an important tool for epidemiological research in sarcocystosis. In several studies, this test was conducted, with special emphasis on the identification of species using sequencing and restriction fragment length polymorphism (RFLP) analysis of 18S rDNA (Fischer & Odening, 1998; Li *et al.*, 2002; Medrano *et al.*, 2006). In addition, in a study where cattle samples were obtained from slaughterhouses, genomic DNA was extracted and analysed by *18S* and *cox1* PCR, obtaining *Sarcocystis* DNA from 82.7% of the samples; where sequence identities of ≥ 97% were observed for *S. cruzi* (65.4%), *S. hominis* (12.5%), *S. bovifelis* (8.7%), *S. hirsuta* and S. heydorni (both 1.0%) (Hoeve *et al.,* 2019).

In the same way, the indirect ELISA test has been reported as a serological diagnostic method, when standardised with the corresponding antigens, it shows a high level of sensitivity, being the test of choice compared to PCR, since its detection time is greater, taking into account that this study was only performed with 15 samples (Medrano *et al.*, 2006). Likewise, a underrecognised diagnostic method called compression analysis has been reported. It consists on taking a muscle sample, compressing it between two glass plates of a trichinoscope until they are translucent, this way they can be analysed under a microscope at a magnification of 100×, where *Sarcocystis* cysts are observed (Luzón *et al.*, 2015).

Regarding the diagnostic method for identification of *Sarcocystis* in humans, first, it should be noted that the symptoms described above should be present and the patient should have a history of consumption of raw or undercooked meat, first we have to know for sure if the sinterstitial sarcocystosis is the reason for the symptomatology already described, although in some cases the people can be asymptomatic (Lindsay & Weiss, 2004; Fayer *et al.,* 2015). Confirmation requires the identification of oocysts and/or sporocysts in the faeces. Sporocysts with size from 10 μm to 15 μm can be easily seen with light microscope in a wet preparation, just below the coverslip with a drop of liquid aspirated from the surface of a faecal float, then it is observed by fluorescence microscopy. Flotation is performed by mixing the faeces with concentrated solutions of zinc sulfate, sucrose, sodium chloride or cesium chloride, Percoll or similar high-density solutions, followed by centrifugation at 500 rpm to sediment the faecal waste while concentrating the parasites on the surface (Fayer *et al.*, 2015).

On the other hand, a faecal suspension can also be made using a portion of 1 g of faeces in distilled water and then centrifuged at 1,500 revolutions for 5 minutes, then a sugar flotation technique is performed using the obtained sediment. A drop of the supernatant is taken and examined under microscope (Pena *et al.,* 2001).

TREATMENT

According to the literature, no prophylaxis or specific therapeutic treatment for intestinal sarcocystosis in animals or humans has been developed (Fayer *et al.*, 2015). A volunteer who ingested sarcocysts of *S. suihominis* was treated with acetylspiramycin for 15 days at 0.2 g dose four times a day, but excretion did not stop until 30 days later (Li *et al.*, 2007). On the other hand, in Malaysia patients with the disease were administered cotrimoxazole at a dose of 2 × 960 mg/day, for 10–20 days, identifying a shorter duration of symptoms compared to previously treated patients (Slesak *et al.*, 2015).

Similarly, there is still no recommendation for treatment of muscular sarcocystosis (Esposito *et al.*, 2015); it is estimated that treatment with albendazole alone or in combination with prednisone may decrease symptoms in some cases of muscle sarcocystosis in humans (Dennis *et al.*, 2013). However, toltrazuril was used for treatment of dogs with sarcocystosis, showing effectiveness three days after the start of treatment and finally, the parasitic infection was controlled (Barrientos *et al.*, 2007).

ECONOMIC IMPORTANCE

Sarcocystosis is a parasitic infection of great importance at clinical and zootechnical and therefore economic level, it has a worldwide high prevalence due to its high percentage of morbidity and mortality in animals' production (Damriyasa *et al.*, 2004; Inga, 2014). Its symptoms result in great losses for farmers. Problems such as abortions, weight loss, low milk production and low quality of wool in sheep are causes for not achieving a good price the obtained products from these animals and also loss of important animal units.

As there is no yet an effective treatment that stops the parasitic infection, farmers are forced to choose sacrificing the animal. Besides that, the muscular sarcocystosis entails condemnation of the carcass in cattle, sheep and pigs, due to the presence of *Sarcocystis* in the meat for human consumption and its zoonotic po-

tential, which means the farmer does not obtain a profit for the sacrifice of the animal (Poulsen & Stensvold, 2014).

CONCLUSIONS

This review made clear that *Sarcocystis* spp. is a parasite with high zoonotic potential, which is distributed worldwide. There's a lack of deeper research on this parasite; for this reason, it is recommended to carry out more studies to find possible effective treatments and decrease significantly the symptoms in infected patients, as well as specific treatments against *Sarcocystis* spp. in animals. In addition, it is necessary to conduct epidemiological studies at a national level to determine exactly which are the most affected country regions by this parasitic infection and establish mechanisms for control and prevention.

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