

## Review article

# The present status of *Sarcocystis* spp. and sarcocystosis in Iran: a literature review

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**ABSTRACT.** Sarcocystosis due to *Sarcocystis* species, is prevalent among livestock in most parts of Iran, the predominant species being *S. cruzi*, and then followed by *S. hirsuta* and *S. hominis*. Studies on the prevalence and geographic distribution pattern of infection in the most common sites of infection have revealed infection rates up to 100% in at least one of the examined tissues in the country. Although human intestinal and gallbladder *Sarcocystis* infections have been reported, nothing is known about muscular *Sarcocystis* infection in human in Iran. The main aim of this review is to estimate the prevalence of *Sarcocystis* infections in ruminants, dogs, cats, and poultry as well as humans based on the studies conducted in Iran from November 1974 to October 2020. PubMed, Scopus, Web of Science, Science Direct, and Google Scholar, as well as one Persian electronic databases (SID) were searched systematically from November 1974 to October 2020. Publication searches were performed by various combinations of the following terms: “sarcocystosis” or “*Sarcocystis* spp.” and “Iran”. The reference list of selected articles was also manually screened, and the searching process resulted in the selection of 56 studies. The abstracts of the papers published at the congresses were not reviewed.

**Keywords:** *Sarcocystis*, sarcocystosis, sarcosporidiosis, Iran

## Introduction

Sarcocystosis (syn. sarcocystitis, sarcosporidiosis) is defined as infection with the genus *Sarcocystis*, which unlike other coccidian parasites, *Sarcocystis* spp. have an obligatory two-host (prey-predator) life cycle in which the sexual phase takes place in the intestinal wall of carnivores, and the asexual, extraintestinal phase occurs in the tissues and muscles (sarcocysts, Miescher’s tubes) of various intermediate hosts, mostly herbivores [1,2]. In the definitive hosts, the ingested sarcocysts contain thousands of cystozoites which are released in the small intestine, enter the mucosa and parasitize non-epithelial cells in the lamina propria. Development of the cystozoites follows a differentiation into macro- and microgametocytes which are fused to produce a zygote, and develop into an oocyst with two sporocysts, each mature

sporocyst contains four sporozoites. The oocyst wall is thin and often is not detectable, or has already ruptured, releasing the sporocysts. The diagnosis is established by detection of sporulated sporocysts (14.5–17×9–11µm) in the stools [3].

The intermediate host ingests the fully mature (sporulated) sporocysts passed with the definitive host’s faeces and the sporozoites are released in the gastrointestinal tract. They penetrate the intestinal wall via blood and are distributed throughout the tissues and enter the endothelial cells of medium and small-sized blood vessels of many organs. The asexual phase occurs in endothelial cells, sometimes resulting in obvious arteritis. The cystozoites produced during the last cycles enter the heart, skeletal muscle or both and develop into a sarcocyst containing thousands of cystozoites [1,2]. Depending on the species of *Sarcocystis*, humans may serve as definitive or accidental intermediate hosts [2].

According to the obtained results of other studies performed in different countries, the prevalence of *Sarcocystis* spp. among ruminants varies in different parts of the world. For example, this rate has been reported among sheep as 97% in Iraq, 93% in Ethiopia, 90% in Turkey, and 52.51% in China [4]. In this regard, *Sarcocystis* spp. have a prevalence rate of almost 100% in the muscles of adult bovine in the majority of regions across the world (e.g., 99.6% in New Zealand, 97.8% in Iraq, 97% in Belgium, and 82.4% in Ethiopia) [4]. The prevalence rates of *Sarcocystis* infections in buffaloes have been reported as 100% in Thailand, 87% in India, 82.9% in Iraq, and 79% in Vietnam [4]. The overall prevalence of this parasite in camels is 100% in Mongolia, 91.6% in Iraq, 88.4% in Saudi Arabia, and 81% in Sudan [4].

The main aim of this review is to estimate the prevalence of *Sarcocystis* infection based on the studies conducted in Iran from November 1974 to October 2020.

Most of the other review articles dealt with this infection only in ruminants, but in addition to ruminants, this review article also dealt with the prevalence of this infection in carnivores and humans.

### Literature search, inclusion, and exclusion criteria

The databases and search engines employed for the literature review including five English language electronic databases (PubMed, Scopus, Web of Science, Science Direct, and Google Scholar) and one Persian electronic database (SID) were searched systematically from November 1974 to October 2020. Publication searches were performed by various combinations of the following terms: “sarcocystosis” or “sarcocystitis” or “sarcosporidiosis” or “*Sarcocystis* spp.” and “Iran”. The reference list of selected articles was also manually screened, and the appropriate articles were included. The abstracts of the papers published at the congresses were not reviewed because they did not have enough details for quality assessment. Dissertations and theses were not included. The searching process resulted in the selection of 56 studies.

### Infection in ruminants

High prevalence of *Sarcocystis* infection has

been reported among cattle and sheep in various parts of Iran (Tab. 1).

#### Cattle

Based on published articles, ruminants especially cattle are common and suitable intermediate hosts for *Sarcocystis* spp. and as one of the most important food sources for humans in Iran [4,5]. *Sarcocystis* spp. have a prevalence rate of almost 100% in carcasses of adult bovine in the majority of regions in Iran [6–8]. The three species, *S. cruzi*, *S. hirsuta*, and *S. hominis* have been observed in cattle, with *S. cruzi* being detected as the most common species with the highest pathogenicity in Iranian bovine sarcocystosis [8–13]. The first identification of *S. cruzi* and *S. hominis* from cattle using PCR-RFLP was done in Babol, northern Iran and Yazd, central Iran, respectively [14,15]. The first global detection of *S. gigantea* in cattle was also reported in Iran [13]. Amongst the *Sarcocystis* spp. present in Iranian cattle, *S. cruzi* and *S. hominis* are capable of transmission to humans [3,16].

#### Sheep

Overall, among ruminants after camel, sheep have the least prevalence of *Sarcocystis* spp. Amongst sheep, the prevalence of *Sarcocystis* spp. varies in different parts of Iran [17–24]. Many *Sarcocystis* spp. have been observed in sheep [25,26] with *S. ovis* as the most pathogenic species of *Sarcocystis* that was reported in the slaughtered sheep’s brains in North Khorasan province for the first time [27].

#### Goat

The prevalence of *Sarcocystis* spp. in goats is the highest prevalence after cattle in different ruminants in Iran [28] (Tab. 1). Although, so far, three species of *S. capracanis*, *S. hircicanis*, and *S. moulei* have been identified in goats in different parts of the world, in Iranian goats, only *S. moulei* has been reported in Tehran and Qazvin provinces [29] (Tab. 1).

#### Water buffalo

Like cattle, water buffalo (*Bubalus bubalis*) is one of the important meat sources in Iran. Overall, among Iranian water buffalo, four species of the genus *Sarcocystis*, including *S. dubeyi*, *S. fusiformis*, *S. buffalonis*, and *S. levinei*, have been reported, only in Khuzestan Province [10,21,30] (Tab. 1).

Table 1. Literature review of Iranian ruminant carcasses infected with *Sarcocystis* spp.

Host/Reference	Province	Total sample	No. of positive (%)	<i>Sarcocystis</i> spp.	Diagnostic methods
<b>Cattle</b>					
[6]	Kerman	480	480 (100)	ND	Naked eye
[21]	Khorasan	45,360	0 (0)	ND	Naked eye
[9]	Isfahan	100	92 (92)	<i>S. cruzi</i> , <i>S. hirsuta</i> , and <i>S. hominis</i>	Histopathology
[19]	Khuzestan	125,593	0 (0)	ND	Naked eye
[10]	Khuzestan	124	124 (100)	<i>S. cruzi</i>	Pepsin digestion, PCR-RFLP
[55]	Alborz	125	121 (96.8)	<i>S. cruzi</i>	Histopathology
[11]	Fars	101	91 (90.9)	<i>S. cruzi</i> , <i>S. hominis</i>	Histopathology, PCR-RFLP
[7]	East Azarbaijan	670	670 (100)	ND	Histopathology
[12]	Khuzestan	25	25 (100)	<i>S. cruzi</i>	PCR-RFLP
[4]	Sistan and Baluchestan	500	441 (88.2)	ND	Histopathology
[8]	Sistan and Baluchestan	100	100 (100)	<i>S. cruzi</i> , <i>S. hirsuta</i> , and <i>S. hominis</i>	Histopathology
[13]	Northwest Provinces	290	267 (92)	<i>S. cruzi</i> , <i>S. hominis</i> , and <i>S. gigantea</i>	Histopathology, PCR-RFLP
<b>Sheep</b>					
[17]	Major sheep raising areas	6,120	6,072 (99.21)	ND	Naked eye, Histopathology
[18]	Fars	1362	786 (57.7)	<i>S. gigantea</i> , <i>S. medusiformis</i> , and <i>S. tenella</i>	Histopathology
[21]	Khorasan	275,439	(0.04)	ND	Naked eye
[19]	Khuzestan	1,191,871	1	ND	Naked eye
[22]	Isfahan, Qom, Shahr-kord	325	299 (92)	ND	Pepsin digestion
[56]	Khorasan	40	40 (100)	<i>S. gigantea</i> , <i>S. tenella</i>	PCR-Sequencing
[25]	West Azarbaijan	638	235 (36.83)	<i>S. gigantea</i> , <i>S. medusiformis</i>	PCR-RFLP
[26]	Khozestan and Lorestan	40	40 (100)	<i>S. gigantea</i>	PCR-RFLP
[23]	Yazd	70	70 (97.14)	ND	Pepsin digestion
[27]	North Khorasan	80	1 (1.25)	<i>S. ovicanis</i>	PCR-Sequencing
[57]	Mazandaran	150	50 (33.3)	<i>S. gigantea</i> , <i>S. moulei</i>	PCR-Sequencing
[24]	East Azarbaijan	620	21 (3.3)	ND, <i>S. tenella</i>	Pepsin digestion and Histopathology
[12]	Khuzestan	25	20 (80)	<i>S. capracanis</i>	PCR-RFLP
<b>Goat</b>					
[28]	Fars	169	169 (100)	ND	Pepsin digestion
[21]	Khorasan	115,674	0 (0)	ND	Naked eye
[19]	Khuzestan	240,221	0 (0)	ND	Naked eye
<b>Buffalo</b>					
[30]	Khuzestan	100	83 (83)	<i>S. buffalonis</i> , <i>S. levinei</i> , <i>S. dubeyi</i>	Histopathology
[19]	Khuzestan	25,010	58 (0.23)	ND	Naked eye
[10]	Khuzestan	147	40	<i>S. fusiformis</i>	PCR-RFLP

ND: not determined

### Camels

Studies on the prevalence of infection in Iran using histopathological examination revealed infection rates between 51.5% and 83.6% in at least one of the camels' examined tissues [31–34]. Although, nothing is known about *Sarcocystis* infection in Bactrian camels (*Camelus bactrianus*) in this country, in ultrastructural and molecular characterization studies of *Sarcocystis* isolated from *Camelus dromedarius*, *S. cameli* was identified [35].

### Dogs and cats

*Sarcocystis* spp. occur among dogs and cats in various areas indicating the cause of the muscular sarcocystosis among livestock in this country. For example, stool examination of 112 stray cats and 147 pet dogs revealed oocysts/sporocysts of *Sarcocystis* in 2.04% of dogs in Shiraz, Fars Province [36]. In another study, the percentage of stray cats infected with the parasite in Ahvaz, Khuzestan Province, south-west of Iran was 17.1% [37]. In a study from different geographic regions of Kashan, 8% of 113 stray cats at necropsy were infected [38]. Dogs' infection is also found in the northwestern Iran (Zanjan Province), where a prevalence of *Sarcocystis* of 7.3% faecal samples examined has been reported [39]. Faecal examination of 28 domestic and 49 stray dogs by Beiromvand et al. [40] in 17 villages from the Chenaran County, Khorasan Razavi Province indicated that 17% of dogs are infected.

### Poultry

For the first time, the study performed by Khordadmehr et al. [41] revealed that two of 94 pigeons with sarcocystosis and nematode disease (*Hadjelia truncata*) in Yazd province were co-infected. Also, for the first time, a microscopic examination revealed tissue cysts of *Sarcocystis* in 96.5% of 57 native birds (ducks and chickens) using digestion method and by light microscope in Mazandaran, north part of Iran [42].

### Boar's infection

The first molecular report of *S. miescheriana* from the thigh muscle of a captured wild boar (*Sus scrofa*) was done from Gilan Province in northern Iran [43]. Due to religious restrictions, the

consumption of pork is prohibited, so there have been no reports of human infection with *S. suis*.

### Meat products

Ahmadi et al. [44] described the first report of molecular identification of *S. hominis* in a sample of raw traditional hamburgers using PCR-RFLP in Yazd, central Iran. In the same region, PCR-based examination revealed *S. cruzi*, *S. hirsuta* and *S. hominis* in 77.9% of raw hamburger samples (100 traditional and 90 industrial) [45]. The infection rate in the traditional hamburgers was significantly higher than the industrial ones ( $P < 0.05$ ). Dehkordi et al. [46] detected the parasite in 80% of a total of 20 samples of hamburger, sausage, and cocktail collected from markets in Hamedan by digestion and impression smear methods. In a molecular research, 29% of the 200 raw industrial hamburger samples with at least 60% meat in Kashan, central Iran were found to be infected with *S. cruzi*, *S. hirsuta*, and *S. hominis* [47]. In a microscopic study, Nematollahia et al. [48] found that 56.3% of a total of 96 industrial and traditional hamburger samples in Tabriz, northwest of Iran were infected with cyst of *Sarcocystis*. In a study conducted [49] on 80 original hamburger samples collected from Garmsar retail food stores, the results of histological methods showed that 6.25% of the tissue samples stained with hematoxylin and eosin were infected. Some photomicrographs showed more than one cyst at a microscopy field and they had various sizes. Amongst 25 commercial hamburger samples collected from supermarkets of Yazd [50], 17 samples showed a PCR product which could detect *S. hominis* or *S. hirsuta*, and detected *S. cruzi* using restriction fragment length polymorphism (RFLP) analysis.

### Human infection in Iran

Although the genus *Sarcocystis* species are widespread among cattle, sheep, and goats, to date only eight cases of human intestinal infections have been reported in Iran. The first case in Iran was found in a woman in the rural area of Babol on the Caspian Sea Littoral [16]. Other cases have been reported in four among 23,875 persons examined in Fars Province, the southwestern Iran [51]. The only documented case of *Sarcocystis*-associated acalculous cholecystitis in an Iranian patient with

HIV/AIDS has been reported by Agholi et al. [52,53]. As mentioned above, although there have been several cases of intestinal *Sarcocystis* infection in humans in Iran, one molecular confirmed case of intestinal infection owing to *S. cruzi* has been reported [3]. In an unconfirmed microscopic report by molecular method, an organism morphologically similar to merozoites of *Sarcocystis* in the peripheral blood smear of an immunocompetent woman was demonstrated [54]. Nothing is known about muscular *Sarcocystis* infection in human in Iran.

## Discussion

*Sarcocystis* infection of livestock is common in ruminants in Iran. In some reports in Iran, almost 100% of the slaughtered cattle have sarcocysts in their muscles [12,55]. The main species in cattle being *S. cruzi*, and then followed by *S. hirsuta* and *S. hominis* in cattle [8,10,12,55]. Overall, the weighted prevalence of *Sarcocystis* spp. among sheep is low (63.83%) compared with other ruminants in different regions of Iran, which is the least prevalent among ruminants after camel [4]. The prevalence of *Sarcocystis* spp. among goats in Iran is relatively high (82.36%) [4]. *S. gigantea*, *S. medusififormis*, *S. ovicanis* (in brain samples) [27], and *S. tenella* in sheep [12,56] and *S. moulei* in sheep and goat [29,57], *S. miescheriana* in the wild boar [43], *S. cameli* in camel [32,35], *S. cruzi*, *S. fusiformis*, *S. buffalonis*, *S. dubeyi*, and *S. levinei* in water buffalo (*Bubalus bubalis*) have been reported [10,30,58].

Several factors contribute to the high prevalence in muscle infections in ruminants. Several species may infect a particular ruminant and there may be abundant definitive hosts for each species infecting that host. As there are an estimated 700,000 stray or sheep-dogs in Iran [59,60], it is clear that very large numbers of oocysts/sporocysts of this coccidian parasite are excreted each day onto Iranian soil, including the fields in which grasses are grown. Contamination of pastures and vegetation with this parasite's oocysts/sporocysts are sources to infect herbivores in Iran at the time of grazing. Oocysts/sporocysts remain viable for many months in the pasture, are resistant to freezing, and can overwinter on pasture [2]. The definitive hosts repeat shedding of oocysts/sporocysts occurs each time a meal of infected meat is consumed [2]. Unlike those of many other genera of coccidia,

oocysts/sporocysts of the parasite, are passed in faeces in the infective form freeing them from dependence on warm, moist weather conditions for maturation to infectivity [2].

Oocysts/sporocysts are spread by invertebrate transport hosts to other areas. Shedding of oocysts and sporocysts in faeces of the definitive hosts is the key factor in the spread of *Sarcocystis* infection; to interrupt this cycle, companion carnivores should be excluded from farm animals and from feed, water and bedding for livestock [2]. Uncooked carcasses or offal should never be fed to companion dogs and cats [2]. The carcasses of slaughtered or dead livestock should be removed and condemned or should never be left in the field for vultures and carnivores to consume. Since freezing can completely kill infective sarcocysts, if not cooked, the flesh should be frozen. Exposure to heat at 55°C for 20 minutes kills sarcocysts [2].

According to the results of the reviewed articles, camels are restricted to desert areas with warm climate, therefore, they are not considered a major source of meat products in Iran. Probably due to the low number of studies, type of diagnostic method with different sensitivity and specificity, small carnivorous population in camel-keeping areas, unsuitable weather conditions for the survival of oocysts excreted from canids as the final hosts, and a strong immune system of camels protecting them against infection, among all ruminants in Iran, the camel has a low frequency of *Sarcocystis* infection. So far, only *S. cameli* has been reported to be observed in Iranian camels [35].

Although there have been several cases of *Sarcocystis* infection in carnivores, and in livestock and the parasites' molecular characteristics in Iran, there is a little information on human infection in the country. The problem in determining the prevalence of this infection in humans is that stool examination usually does not show the true image. However, few studies on other intestinal parasites have shown that there are very few human cases of infection in the country [3,51].

A review of studies published in Iran shows that species that use canines as the final host are more common than species that use cats. For example, of the three species in cattle, *S. cruzi*, for which the dog is the definitive host, is the most common, whereas *S. hirsuta* and *S. hominis*, which use cats and humans as definitive hosts, respectively, are less common in the country.

## Research limitations

Some limitations of this study include: (a) the difference in the sample size of the reviewed studies (25–1,191,871 ruminants); (b) use of different diagnostic methods in the studies reviewed with different sensitivities and specificities; (c) limited data about disease severity or weather conditions influencing sarcocystosis; (d) lack of more appropriate diagnostic methods in some of the reviewed studies, such as histopathology and molecular techniques; (e) available data about muscular sarcocystosis is rare in camels and limited to few documents reporting *Sarcocystis* cysts in the *Camelus dromedarius* in Iran.

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