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Original Article

Macroscopic and Microscopic Survey of *Sarcocystis* spp. Infection in Slaughtered Cattle and Sheep in Tabriz, Iran

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Abstract

Background & Aims: *Sarcocystis* infection is one of the most common protozoan infections between humans and animals, which is caused by different species of *Sarcocystis*. The study aimed to investigate *Sarcocystis* infection in cattle and sheep slaughtered in Tabriz slaughterhouse by microscopic and macroscopic methods.

Materials amd Methods: Diaphragmatic muscles of 500 cattle and 800 sheep were randomly selected for macroscopic and microscopic *Sarcocystis* cysts. A naked eye examination was done for macroscopic sarcocysts, while peptic digestion and Daub smear method were used for the microscopic cysts.

Results: The overall prevalence of *Sarcocystis* spp. infection was 44.0% and 68.25% in cattle and sheep, respectively. The results showed that rate of infection of cattle with *Sarcocystis* spp. was 10.4%, 90.0%, and 22.3% by macroscopic, peptic digestion, and Daub smear methods, respectively. Meanwhile, the percentage of infected sheep was determined by macroscopic, peptic digestion, and Daub smear methods as 30.6%, 100.0%, and 44.1%, respectively ($P<0.05$).

Conclusion: It is concluded that digestion is a perfect method for diagnosing sarcocysts in cattle and sheep. As well as, the high prevalence of microscopic *Sarcocystis* spp. in cattle and sheep of Tabriz, is suggested that meat should be cooked sufficiently, the people to be trained not to feed their dogs and cats with uncooked meat.

Keywords: *Sarcocystis*, Cattle, Sheep, Macroscopic, Tabriz, Iran

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1. Introduction

The phylum Apicomplexa's intracellular protozoan *Sarcocystis* is responsible for the zoonotic disease sarcocystosis [1]. This parasite consists of more than 190 species and affects a variety of domestic animals, including cattle, sheep, goats, and pigs. In these animals, the parasite can lead to weight loss, anorexia, lameness, paralysis, fever, anemia, decreased milk production, abortion, and even death [2-4]. Some *Sarcocystis* species are important pathogens of humans and domestic and wild animals. These parasites are characterized by an obligatory two-host life cycle, the formation of sarcocysts mainly in the muscles of the intermediate hosts and endogenous sporulation of oocysts in the intestine of the definitive hosts [2]. Due to the condemnation of contaminated carcasses, millions of dollars of damage are caused to the livestock industry every year, and the disease caused by different species of these protozoa is significant both in terms of health and economics [5-8].

By consuming undercooked or raw infected meat, humans can become definite hosts of *Sarcocystis hominis* and *S. suihominis*, and they are responsible for intestinal sarcocystosis. Most people with intestinal sarcocystosis remain asymptomatic. Symptoms induced by experimental infections include nausea, abdominal discomfort, and self-limited diarrhea, with symptom severity varying with the amount of meat consumed [9]. Diarrhea usually onset suddenly (in some people 3 to 6 hours after consumption; usually within 48 hours) and symptoms resolve within 36 hours. They can also become intermediate hosts of *S. nesbitti* and *S. lindemanni* by consuming sporocysts excreted by reptiles or carnivores, which can cause extraintestinal sarcocystosis and symptoms such as musculoskeletal pain, fever, nausea, abdominal pain, diarrhea, and cardiopathy [9,10]. To date, several *Sarcocystis* species, including *S. medusiformis* and *S. gigantea* (*S. ovifelis*, which has macroscopic cysts), and *S. arieticanis* and *S. tenella* (*S. ovicanis*, which has microscopic cysts), have been linked to sheep [2,11,12]. *S. cruzi*, *S. hirsute*, and *S. hominis* are the three *Sarcocystis* species found in cattle, with dogs, cats, people, or primates serving as the three definitive hosts, respectively [13,14].

Some species of *Sarcocystis*, particularly those having a canid as the definitive host, may cause clinical signs/symptoms including fever, inappetence, abortion, stillbirth, or central nervous signs including acute myopathy, ataxia, paresis, and death in sheep and cattle [15-17].

Those signs have been associated with the release of interleukin-1 (IL-1), prostaglandin E2 (PGE2), and tumor necrosis factor–alpha (TNF-α) by the *Sarcocystis* infected macrophages. These cytokines can cause inappetence, anemia, and suppression of the release of pituitary growth hormone (GH) as a potential reason for weight loss [18].

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The frequency of *Sarcocystis* spp. in sheep and cattle in various regions of Iran and other nations has been the subject of many investigations [19-24]. By using microscopic and macroscopic techniques, this study's goal was to assess the prevalence of *Sarcocystis* infection in the diaphragm muscle of cattle and sheep that were slaughtered for food in Tabriz, Iran.

2. Materials and Methods

From March 2018 to March 2019, the diaphragmatic muscles of 500 cattle and 800 sheep were inspected for macroscopic and microscopic *Sarcocystis* cysts. Animals were examined at arrival by veterinarians in accordance with all the veterinary criteria scheduled for the antemortem examination included in Iran's legislation [25]. The absence of clinical symptoms was established according to the antemortem veterinary official examination.

The number of samples was calculated using the following formula, assuming a prevalence of 20-30% of *Sarcocystis* infection in animal meat based on previous studies, as well as a 96% confidence interval and an accuracy of 5%.

$$n = \frac{z^2_{1-\alpha/2} \times p(1-p)}{d^2}$$

Where P: Prevalence, α: Error rate, d: Accuracy

A naked eye examination was done for macroscopic sarcocysts, while peptic digestion and the Daub smear method were used for the microscopic cysts [26]. For this purpose, diaphragmatic muscles that apparently lacked *Sarcocystis* cysts resembling rice grains were selected. About 1 g of diaphragmatic muscles were broken up into little pieces and firmly compressed between two glass slides for the Daub smear procedure. Then the samples were examined under a microscope after staining with Giemsa. In the digestion procedure, 50 mL of digestion solution containing 2.5 g pepsin (Sigma-Aldrich, Germany) and 10 mL of HCl (Merck, Germany) in 500 mL of distilled water, was added to 10 g of crushed muscles and heated for 4 hours at 40 °C. The solution was filtered through a strainer, and centrifuged for 2 minutes at 1500 g. The supernatant was decanted, the sediment was resuspended in 1 mL of Giemsa saline, prepared a smear, and observed under a compound microscope at 400× magnification [2]. Data were analyzed by SPSS software (version 21) and chi-square test analysis. A significance level of less than 0.05 was considered.

3. Results

The animals studied in this study were apparently healthy and had no clinical symptoms. The results of this study showed that the prevalence of *Sarcocystis* spp. infection in sheep (68.25%) was significantly higher than in cattle (44.0%) ($P<0.05$).

The prevalence of microscopic *Sarcocystis* spp. cysts in cattle were detected in 90.0% and 22.3% using peptic digestion and Daub smear, respectively, while 10.4% were detected in macroscopic examination (Table 1).

Two hundred and forty-five of the 800 sheep (30.6%) were diagnosed as infected with macroscopic cysts. Out of 100 diaphragmatic muscles of sheep examined using the digestive method, all samples were infected with *Sarcocystis* cysts. In addition, out of 455 samples tested by the Daub smear method, 201 samples (44.1%) were found to be infected.

The results of this study revealed that the prevalence rate of infection with *Sarcocystis* spp. in females was higher than in male animals ($P<0.05$). Also, the results of the comparison of different methods in the diagnosis of *Sarcocystis* spp. infection in cattle and/or sheep showed that the digestive method was better than macroscopic and/or Daub smear methods.

4. Discussion

One of the most widespread protozoan zoonotic parasites, *Sarcocystis*, is found in the striated muscles of livestock killed for food, such as cattle, sheep, and goats. It poses serious health and economic risks to both human and animal civilizations [15,26]. In slaughterhouse inspections, only macroscopic cysts are detected, and microscopic cysts remain hidden from the inspectors. Therefore, the statistics presented by the slaughterhouse inspection are less than the real value [27].

In the present study, the overall prevalence rate of *Sarcocystis* spp. in sheep was higher than that in cattle, which was consistent with other studies [28-31]. It may relate to different forage habitats of cattle and sheep, geographical climates, or high exposure to dogs and/or cats, which serve as definitive hosts [32].

Table 1. Infection of *Sarcocystis* spp. in sheep and cattle slaughtered at Tabriz abattoir on the sex by different methods

Method/ Animal	Cattle					*P* value	Sheep					*P* value
	No. examined		No. positive samples (%)		Total (%)		No. examined		No. positive samples (%)		Total (%)	
	M	F	M	F			M	F	M	F		
Macroscopy	250	250	15 (6)	37 (14.8)	52 (10.4)	0.000	400	400	100 (25)	145 (36.2)	245 (30.6)	0.000
Peptic digestion	50	50	45 (90)	45 (90)	90 (90)		50	50	50 (100)	50 (100)	100 (100)	
Muscle squeeze	185	163	32 (17.29)	46 (28.22)	78 (22.3)		250	205	88 (35.2)	113 (55.1)	201 (44.1)	
Total	250	250	92 (36.8)	128 (51.2)	220 (44)		400	400	238 (59.5)	308 (77)	546 (68.25)	

The results of this study showed that the prevalence rate of infection with *Sarcocystis* spp. in females was higher than in male animals ($P<0.05$), which was in line with the studies conducted by Mac et al [22]. However, there was no significant association between sex and *Sarcocystis* spp. infection in cattle [22]. This finding may be due to the higher mean age of females than males, with females being slaughtered at an older age [33].

By using macroscopy, peptic digestion, and Daub smear methods in the current investigation, the prevalence of *Sarcocystis* spp. in cattle was found to be 10.4%, 90.0%, and 22.3%, respectively. Similar results were obtained by Rasouli et al [34], who found an infection rate of 93.33% by pepsin digestion in Kurdistan, by Nourani et al [35], who reported an infection rate of 92% by histopathological in Isfahan.

In the study conducted by Mirzaei and Rezaei [36], the prevalence of microscopic and macroscopic *Sarcocystis* spp. cysts in cattle of Tabriz city, Iran were detected in 100.0% and 8.2%, respectively. In various reports from different regions of Iran, the infection prevalence rate is 100.0% using the pepsin digestion method, which can be mentioned in the following studies: Nourollahi-Fard et al [26] in Kerman, Dalimi Asl et al [37] in Tabriz, Parandin et al [38] in Hamadan, Rahdar and Salehi [39], Hamidinejat et al [40] in Ahvaz, and Shekarforoush et al [4] in Shiraz.

The prevalence rate of *Sarcocystis* spp. by the pepsin digestion method was estimated to be 59.8% in Bukan [34] and 26.42% in Saqez [27], which was lower compared to our results.

However, the prevalence rate obtained in the recent study was lower compared to some studies conducted by other researchers. For example; Bonyadian and Meshki [41] showed an infection rate of 91.0% by Daub smear in Shahrekord.

Hamidinejat et al [40] reported the prevalence of *Sarcocystis* infection by digestive and Daub smear methods as 100.0% and 94.7% respectively. In another study, of the 140 inspected cows, 27 (19.28%) were infected with *Sarcocystis* spp. by Daub smear [27].

The prevalence rate of *Sarcocystis* spp. in other countries has been reported as follows: Hungary (66.0%) [42], Southern Italy (96.0%) [43], and Nigeria (42.5%) [44].

Our study's findings, which were in line with other research, showed that the prevalence of *Sarcocystis* in slaughtered sheep was 30.6%, 100.0%, and 44.1% by macroscopic, digestive, and Daub smear techniques, respectively ($P<0.05$). Daryani et al found the prevalence of *Sarcocystis* infection in sheep slaughtered in Ardabil to be 33.93% by macroscopic method [21], and Arshad et al [45] in Tabriz and Rahdar [24] in Ahvaz reported 100.0% by peptic digestion method. The frequency of *Sarcocystis* infection was determined to be 6% in sheep slaughtered in Kerman, according to Mirzaei Dehaghi et al [7].

The prevalence of *Sarcocystis* spp. in sheep varies in different parts of the world. In this regard, this rate has been reported as 92.5% by digestive method [46] and 97.0% and 4.10% by digestive and macroscopic method, respectively in Iraq [47], 52.51% by digestive method in China [48] and 100.0% by digestive method in Lithuania [31].

The reasons for the difference in the level of *Sarcocystis* infection in different regions of the world come from the fact that there are important factors in the transmission and high frequency of this parasite in ruminants. Some of these factors include close contact of animals with carnivores, especially dogs and cats, type of feeding that is mostly traditional grazing (final hosts infected with this parasite having access to these pastures and transmitting this parasite to ruminants), the viability of *Sarcocystis* sporocysts for many months in the environment and weather conditions suitable for the survival of oocysts in the environment [32].

Since these cysts are of feline origin and in Tabriz there is more contact between these animals and dogs in pasture than there is between those animals and cats, the low prevalence of microscopic sarcocysts in sheep and cattle in the current study may be the cause [28].

The gold standard method for diagnosing sarcocysts is pepsin digestion, and research in sheep or cattle found that it was more sensitive than the muscle squash method [40].

5. Conclusion

In the present investigation, most cysts in sheep and cattle are microscopic, and the final host is the dog, and the contact between the sheep and cattle and the herd dogs is high and has caused high infection of livestock, and since it is done macroscopically, the inspection of the carcasses in slaughterhouses, therefore, it is necessary to change the method of inspection and control of meat in slaughterhouses, as well as to decide on special methods to eliminate meat contamination before consumption to prevent human infection.

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Competing Interests

The author declares no conflicts of interest relevant to this study

Ethical Approval

The study entailed recording of the normal meat inspection process of animals sent to slaughter for human consumption. No ethical approval was required nor sought as no alteration was required to the normal processing of these animals.

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