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A study on the abundance of infection with *Sarcocystis* in goats slaughtered in the Tabriz slaughterhouse

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ABSTRACT

Sarcocystis is a mandatory intracellular parasite that is common between humans and animals, which causes huge losses in the livestock and meat industries. This study aimed to determine the prevalence of *Sarcocystis* infection in goats slaughtered in Tabriz slaughterhouse. For this purpose, the carcasses of 240 slaughtered goats were examined for the presence of macrocysts by eye observation and then by microscopic by dob-smear and digestive methods. Based on the results, 2.5% of the studied carcasses were diagnosed with macrocytic observation. In the microscopic examination by dob-smear and digestion methods, 35.83% and 100% respectively of the cases were positively diagnosed. There is a significant relationship between contamination and the studied method ($p<0.05$), which has higher validity digestion than the dob-smear and macroscopic method. There was also a statistically significant difference between the contamination rate among different muscles ($p<0.05$). As well, the highest microcyst contamination was observed in diaphragm muscles and the lowest microcyst contamination was observed in the heart muscle. This study showed the necessity of microscopic observation of the *Sarcocystis* contamination in the goat carcasses.

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1. Introduction

Sarcocystis is currently one of the most common parasites in domestic animals. Infection with this parasite is very severe in some hosts such as cattle, sheep, and goats. Some species of *Sarcocystis* can cause disease with symptoms such as weight loss, anorexia, lameness, paralysis, fever, anemia, muscle weakness, decreased milk production, miscarriage. Death has also been seen in intermediate hosts such as cattle, sheep, goats, and pigs due to infection with this parasite (1-3). Three types of *Sarcocystis* were reported in domestic goats including, *S.hircicanis*, *S.capracanis*, *S.caprafelis*. The first two types cause microscopic cysts and the third type macroscopic cysts.

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The rate of livestock infection with this parasite in different parts of the world is estimated at 70-100% (4). *Sarcocystis* was first reported by Meisher in mice (5), and because this zoonotic parasite is between humans and animals, and according to the rules of some countries, if it is observed in the viscera and muscles of slaughtered animals in the slaughterhouse, it should be removed (6). According to internal and external studies, the meat industry suffers millions of dollars annually as a result of the extermination of *Sarcocystis*-infected carcasses (7). It is also noted that this parasite is very common in Iran, and it has been identified in hamburgers and minced beef Infection in humans occurs when the undercooked meat of an infected animal is eaten by a cyst (8). Some species of *Sarcocystis* in humans cause digestive disorders such as nausea, vomiting, diarrhea, an increase in heart rate, and difficulty breathing (9). Therefore, its control is important in terms of public health.

Since the parasite is commonly present in the skeletal muscle and heart muscle of domestic animals such as sheep, cattle, buffalo, goats and can cause intestinal infections in humans (10) most human infections are due to the ambiguity of the effects and complications, And the inaccuracy and familiarity of doctors are not diagnosed. Therefore, studying the prevalence of this parasite in livestock, while it can increase awareness and the possibility of timely prevention in preventing livestock casualties, can certainly lead to more familiarity with the infections caused by this parasite in humans and also the complications and damages caused by it.

Field studies have shown that slaughterhouses usually detect only macroscopic cysts in slaughterhouses, and microscopic cysts remain hidden from meat inspectors, so the statistics provided by slaughterhouses are less than the actual value. Due to the contamination of small ruminants such as goats in the country and East Azarbaijan province and Tabriz city as one of the most important cities in the province and where slaughtering animals such as goats is common, it is necessary to conduct a macroscopic study at the slaughterhouse. Microscopic examination of *Sarcocyst* prevalence in slaughtered goat muscles should also be performed. This study aimed to determine the prevalence of *Sarcocystis* infection in goats slaughtered in the Tabriz slaughterhouse by comparing the three methods of carcass inspection, dob-smear method, and digestion in the diagnosis of meat infection with this parasite. The novelty of the present study was to examine cysts in different ways and compare their results with each other.

2. Materials and Methods

2.1. Sampling

This descriptive cross-sectional study was conducted during the two-years from October 2019 to March 2021. During the implementation of the project, a total of 240 carcasses of slaughtered goats were examined macroscopically. After preparing the studied carcasses, different tissues including the thigh, arm, heart, diaphragm, and esophagus muscles were observed and inspected for the presence of rice grain cysts.

Information about livestock such as genus and tissue containing macrocysts and severity of infection were recorded in the information form. Then, for microscopic examination, 100 g of each animal tissue was packed by microscopic method and transferred to the parasitology laboratory; after preparing 50 g of the sample was hemolyzed and digested and tissue staining was performed to examine micro-cysts in different tissues of each animal.

2.2. Investigation of macroscopic cyst infection

Skeletal muscles, tongue, heart, esophagus, and diaphragm were examined for macroscopic cysts in the slaughterhouse. Also, in the laboratory, the external surface of the samples and then the depth of each sample were examined for the presence of macroscopic cysts by making sheet incisions.

2.3. Investigation of microscopic cyst infection with dob-smear method

Each sample is taken with surgical forceps and pressing on the filter paper, the bloody sample is taken out and then pressed on the slide several times so that a layer of it remained on the slide. The slides were numbered, then fixed with absolute methanol for 3 min, and stained by Giemsa. The slides were then examined for the presence or absence of *Sarcocystis* by light microscopy.

2.4. Investigation of microscopic cyst infection by enzyme hydrolyzes

Sarcocystis infection of tissues was done by Rahdar and Salehi method (11). Fifty grams of each sample, without being heated were ground by blender and digested in 1.5% HCL and 0.5% pepsin solution at 28°C overnight. The digested samples were sieved through the mesh and centrifuged at 1500 rpm for 5 min. After discarding the supernatant fluid, the sediment was stained by Giemsa and examined microscopically for detecting *Sarcocystis* bradyzoite.

2.5. Statistical analysis

SPSS software version 19 (SPSS Inc., Chicago, USA).was used for statistical analysis of data. K-square test ($p<0.05$) was used to determine the relationship between variables.

3. Results

3.1. Investigation of macroscopic cyst infection

Out of 240 samples taken from slaughtered goats, 6 samples (2.5%) were positively detected by the macroscopic method. There were 4 positive samples related to male goats (2.7%) and 2 samples related to female goats (2.17%). The frequency of infection in different tissues was also examined macroscopically. The results are given in Table 1. Esophagus with 4 samples (66.66%), diaphragm 5 (83.33%), arm muscle 4 (66.66%), thigh muscle 3 (50%) were positive cases.

Table 1. Frequency (%) of *Sarcocystis* infection in different sexes of goats in the macroscopic examination.

sex	Number of Samples	Positive Samples	Frequency (%)
Male	148	4[a]	2.7
Female	92	2[a]	2.17

*Values with different letters (a–b) are statistically different.

3.2. Investigation of microscopic cyst infection with dob-smear method

According to the results are given in Table 2. Out of 240 samples, the number of positive cases by this method 86 was with a frequency of 35.83%, of which 54 cases of

male goat carcasses accounted for 36.48% and 32 cases of female goat carcasses accounted for 34.78%. Among the positive samples, esophagus 65 cases (75.58%), heart 46 cases (53.48%), diaphragm 84 cases (97.67%), arm muscle 64 cases (74.41%), thigh muscle 52 cases (60.46%) were positive cases.

Table 2. Frequency (%) of *Sarcocystis* infection in different sexes of goats in microscopic examination.

sex	Number of Samples	Positive Samples	Frequency (%)
Male	148	54[a]	36.48
Female	92	32[a]	34.78

*Values with different letters (a–b) are statistically different.

3.3. Investigation of microscopic cyst infection by Digestion method

All samples were positive for *Sarcocysit* infection in this method. This means that, 148 male and 92 female goats' carcasses, were 100% contaminated.

The numbers of positive cases of tissues are: esophagus 234 (97.5%), heart 216 (90%), diaphragm 240 (100%), arm muscle 236 (98.33%), thigh muscle 235 (97.91%). The results are shown in Fig. 1.

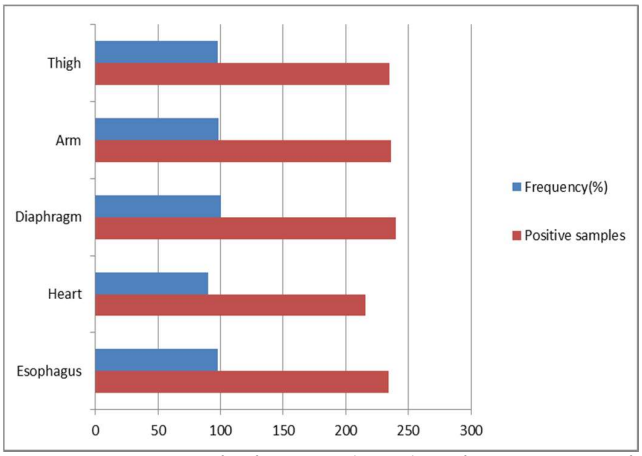

Figure 1. Percentage of infection and number of positive cases of *Sarcocystis* in different tissue

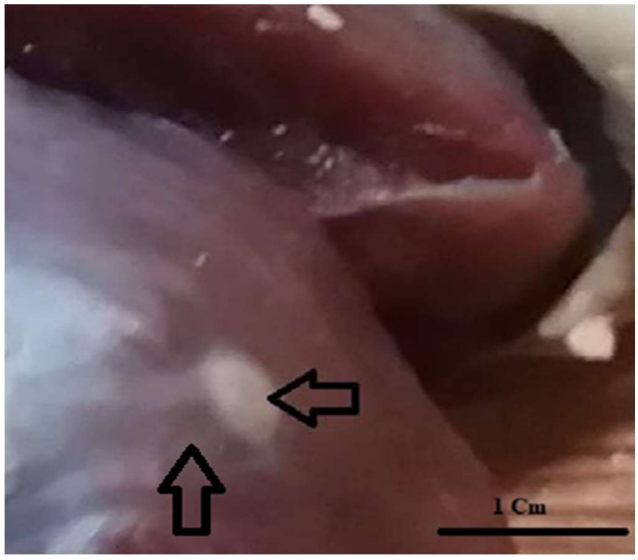

Figure 2. Macroscopic image of sarcocyst parasite in arm muscle

4. Discussion

Sarcocystis is a zoonotic parasite between humans and animals. Infection of various animals, especially ruminants with this parasite in the skeletal and cardiac muscles of animals with the formation of rice grain cysts is significant (Fig. 2). Therefore, controlling the contamination of ruminants with this parasite is important. Examination of the parasite in the tissue by two methods is available: microscopic and macroscopic methods. The microscopic method by digestion method is necessary to detect the presence of the parasite in the tissue. Large cysts of *Sarcocystis* in livestock can be easily identified by slaughterhouse inspection. Because they are visible to the naked eye. However, research has shown that significant cases of infection in small ruminants, such as goats and sheep, cause the production of microscopic cysts and lead to the fact that they cannot be detected by the common method of eye slaughter inspection. The results obtained from this study indicate the existence of

statistical discrepancies between the results obtained from the macroscopic method to the microscopic method ($p<0.05$). So that the positive cases were examined microscopically by 2.5% but microscopic examination by 35.83% and enzymatic hydrolysis were obtained by 100%. Due to the fact that the infection rate with this parasite is high in Iran and other countries. *Berenji et al.,* reported that the frequency of *Sarcocystis* infection in sheep slaughtered in Mashhad slaughterhouse was 34.28% by macroscopic method and 81.90% by histopathology, respectively (12).

In another study conducted by *Ali-Beigi et al.,* to examine macroscopic and microscopic cysts of *Sarcocystis* in slaughtered samples of ruminants, there was no macroscopic cyst but microscopic cysts were positive in 93.48% of cattle and 86.95% of sheep by impression smear method (13). The results of the above studies are in complete agreement with the results of the present study and indicate the superiority of the microscopic method over the macroscopic method.

In the present study, during macroscopic and microscopic examination, there was no statistically significant difference between the sexes of the goats. This indicates that there was no relationship between the prevalence of infection and the age of the animal, which has been obtained in other studies (14,15). The age parameter has not been studied in this study due to some limitations, but the results of the present studies show that there is a relationship between age and the prevalence of *Sarcocyst* parasitic infection. The upward trend in severity of infection in older livestock is likely to be due to longer leisure time and more feeding, resulting in a higher risk of exposure to pastures contaminated with dog feces and sometimes cats, which can carry *Sarcocystis* to livestock (16).

In the study by Valinezhad et al, (17) in Kerman province on the rate of infection of goats with *Sarcocystis*, 98.97% were diagnosed as positive samples by dob-smear method and all 294 samples were positive by the digestion method has been reported. In the present study, *Sarcocystis* macrocyst was found in 2.5% of all samples.

The highest prevalence related to arm muscle and esophagus was similar to the observations of some of the studies that detected the highest prevalence of cyst in the esophagus (18, 19).

5. Conclusion

The results of this study showed that the percentage of *Sarcocystis* infection in goat carcasses slaughtered in Tabriz province is high and since the carcass inspection method detects only macroscopic cysts and is not able to detect microscopic cysts, it is better than dob-smear and digestion methods. Digestion and tissue expansion are used to determine the percentage of true *Sarcocystis* infection. Because macroscopic and microscopic *Sarcocystis* are transmitted through the feces of cats and dogs, respectively, we must keep livestock pastures away from this contamination. Also, despite the high percentage of *Sarcocystis* contamination of goat meat and high consumption of meat in this area, there is a possibility of human contamination, and preventive measures should be taken by relevant organizations. Carcasses are recommended by microscopic and macroscopic methods and complete cooking or freezing of meat.

Conflict of interest

The authors declare they have no conflict of interest.

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