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Prevalence of sarcocystis infection in slaughtered ruminants at Rasht abattoir: A comparative study of digestive and macroscopic methods

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Abstract Detecting zoonotic diseases in slaughterhouse inspections is essential for controlling their transmission to humans. Sarcocystis, a widespread parasitic infection, presents serious public health challenges and causes significant economic losses by necessitating the condemnation of infected livestock carcasses. This study aimed to determine the prevalence of Sarcocystis species infections in slaughtered ruminants (cattle, sheep, and goats) at the Rasht abattoir, Iran, using the digestive method. A total of 606 samples (tongue, esophagus, heart, diaphragm and, skeletal muscle (gluteal muscle)) were examined visually for the presence of macrocysts at the Rasht industrial slaughterhouse. Each animal was categorized based on age, sex, and infected muscle groups. All samples were cut into 2-3 mm pieces and thoroughly observed for potential macrocysts. Subsequently, the samples were analyzed using the digestive method. The results showed that 9.5% of goats, 10% of sheep, and 11% of cattle were infected with macrocysts. Microscopic examination revealed microcyst infection in 23.2% of goats, 21.9% of sheep, and 20.6% of cattle. Although the infection rate did not exhibit a statistically significant correlation with age, a higher prevalence of infection was observed in older animals. (P>0.05). However, infection rates were independent of sex, with no significant difference between males and females (P>0.05). The highest infection rates were observed in the esophageal tissue and diaphragm, at 21% and 17.3%, respectively, while the lowest infection rate was found in the heart tissue, at 9.9%. Our study demonstrated that the digestive method is one of the most useful and accurate techniques for identifying infected samples, as evidenced by our Likelihood ratio assessment.

Introduction

Protozoan parasites of the genus *Sarcocystis* have an obligate two-host life cycle, involving sexual reproduction and oocyst formation in the intestinal mucosa of definitive hosts (herbivores and carnivores) and asexual reproduction in the endothelial cells and striated muscle cells of intermediate hosts (herbivores, omnivores, and carnivores) [11]. Proliferation in striated muscle cells leads to the formation of mature sarcocysts, which have distinguishable morphology (size, shape, and surface structure) for each species. Definitive hosts become infected by consuming contaminated tissues [6]. The definitive hosts spread oocysts through their feces, contaminating the environment, and intermediate hosts become infected by ingesting these oocysts, leading to sarcocystosis [26].

The prevalence of *Sarcocystis* in cattle muscles can reach up to 100% in some parts of the world [21]. Infection with *Sarcocystis* is a

concern in public health and veterinary medicine because it is a zoonotic disease, affecting both humans and animals. Clinical symptoms in humans include nausea, abdominal pain, stomach aches accompanied by diarrhea or loose stools [9]. This protozoan is one of the most common parasites in domestic animals and can cause severe infections in intermediate hosts such as cattle and sheep. Due to the direct contact of these animals with humans, infection in human communities in endemic areas represents a significant public health issue [11].

The diagnostic methods used in epidemiological studies include digestion, histopathology, and examination of fresh slaughterhouse tissues [23]. The artificial digestion method is one of the most sensitive diagnostic methods because it allows the identification of bradyzoites released from sarcocysts. However, this method does not allow the identification of *Sarcocystis* species [26]. *Sarcocystis* is generally non-pathogenic to definitive hosts, and some species are also non-pathogenic to intermediate hosts. Clinical symptoms appear when the second-generation schizonts form in blood vessels (acute phase of the disease). Three to four weeks after infection with a high dose of oocysts (more than 50,000 oocysts), symptoms such as fever, anorexia, emaciation, anemia, and hair loss (especially in the rump and tail of cattle) are observed, although some animals may die. Pregnant animals may experience abortion, or fetal growth may slow down or even stop. Animals recover upon reaching maturity [19].

This protozoan has a global prevalence, and epidemiological reports on *Sarcocystis* have been reported from various parts of the world. Latif et al. conducted a study in 2013 to investigate the prevalence of *Sarcocystis* in cattle and buffalo in Malaysia, where 102 cattle were examined, and 37 cases (36.7%) were reported as positive [18]. Yang et al. reported a prevalence of 41.5% in cattle in China in 2018 [30]. Studies in China have shown a wide range of prevalence, from 17.6% in 2017 (12) to 98.1% in 1990 [29]. High prevalence rates of *Sarcocystis* have also been reported in Mongolia [10], Italy [2], and Vietnam [14]. There are also numerous reports on human infections. In the UK, a patient with arm and leg weakness and blood eosinophilia was reported to be positive for *Sarcocystis* (20). More than 100 human cases where cysts formed in human muscles have been documented in scientific journals [4].

Materials and Methods

This study involved bi-weekly visits to the Rasht slaughterhouse to collect and inspect tissues from 210 sheep, 168 goats, and 228 cattle carcasses, including tongue, esophagus, heart, diaphragm, and gluteal muscle for rice grain cysts. Data on sex, species, age, tissue with macrocysts, and infection intensity were recorded. For microscopic analysis, 100 grams of each tissue were sent to the Parasitology Laboratory at the Faculty of Veterinary Medicine, Islamic Azad University, Urmia. After homogenization, digestion, and staining, microcysts were examined. The data were compiled, classified, and statistically analyzed, with carcasses being sexed and aged through dental examination and reproductive organ inspection.

Macroscopic Inspection

At the slaughterhouse, gluteal muscle, tongue, heart, esophagus, and diaphragm were examined for macroscopic cysts. Additionally, in the laboratory, the external surface of the samples and then the depth of each sample by making thin slices were examined for macroscopic cysts.

Preparation of Direct Smears

In the laboratory, each sample was taken with forceps (ProSciTech, Australia), and after pressing on filter paper to absorb the blood, the sample was imprinted on a slide at least three times to prepare the smear [5]. The sample number was recorded on the slide with a diamond pen (ProSciTech, Australia), and after the smears dried, the slides were placed back-to-back in

Coplin jars and fixed with absolute methanol (Merk) for 3 minutes [8, 16].

Digestion Method

Approximately 50 grams of the desired tissue (tongue, heart, diaphragm, gluteal muscle, and esophagus) were ground using a meat grinder and placed in 50 ml of digestive solution. To prepare the digestion solution, dissolve 2.5 grams of pepsin powder (Merck, Germany) in 100 ml of phosphate buffer (pH = 2.7), and adjust the pH to around 1.5 to 2.0 using 10 ml of hydrochloric acid (Merck, Germany). Incubate the sample container at 40°C (Tajhiz Azma Teb, Iran) for one hour. After digestion, filter the solution through a filter with pore sizes between 40 and 100 micrometers, and centrifuge at 2500 g for 5 minutes (Tajhiz Azma Teb, Iran). Prepare a smear from the sediment, fix it with methanol, and stain with Giemsa. Examine the stained smear under a light microscope at 40x or 100x magnification for the presence of Sarcocystis cystozoites. If no parasites are observed, repeat the test to confirm the result [5].

Microscopic Observation

A light microscope (TOPCON, Japan) with a 100x objective lens was used to examine at least 50 microscopic fields. Positive samples under the microscope contained Sarcocystis cystozoites, which are typically observed in crescent or banana shapes. The presence of even a single parasite classified the sample as positive.

Statistical Analysis

Data processing was performed using SPSS V.19 and Excel V.2013 software. The Chi-square test was used to determine the relationship between variables, and values of $p < 0.05$ were considered significant.

Results

The study analyzed infestation rates across different livestock species, focusing on variables such as age, gender, tissue type, and the type of cysts observed. Below is a detailed analysis of each table and its corresponding data.

The data show that as sheep age, the likelihood of infestation increases. The highest infestation rate is observed in sheep aged 3-4 years (7.1% positive) and the lowest in those under 1 year (1.4% positive).

Table 1. Assessment of infection prevalence by species, age, and gender in ruminants

Species	Variable	Infection		p-value
	Age	Positive (%)	Negative (%)	
Sheep	< 1 year	3 (1.4)	21 (10.0)	
	1- 2year	9 (4.3)	40 (19.0)	
	2-3 year	10 (4.8)	39 (18.6)	
	3-4 year	15 (7.1)	25 (11.9)	
	> 4 year	11 (5.2)	37 (17.6)	
	Total	48 (22.9)	162 (77.1)	0.133
	Gender			
	Male	26 (12.4)	76 (36.2)	
	Female	22 (10.5)	86 (41.0)	
	Total	48 (22.9)	162 (77.1)	0.414
Goats	Age			
	< 1 year	4 (2.4)	22 (13.1)	
	1- 2year	6 (3.6)	26 (15.5)	
	2-3 year	9 (5.4)	26 (15.5)	
	3-4 year	10 (6.0)	31 (18.5)	
	> 4 year	10 (6.0)	24 (14.3)	
	Total	39 (23.2)	129 (76.8)	0.710
	Gender			
	Male	23 (13.7)	58 (34.5)	
	Female	16 (9.5)	71 (42.3)	
	Total	39 (23.2)	129 (76.8)	0.145
Catt	Age			
	< 1 year	4 (1.8)	31 (13.6)	
	1- 2year	7 (3.1)	37 (16.2)	
	2-3 year	12 (5.3)	34 (14.9)	
	3-4 year	11 (4.8)	41 (18.0)	
	> 4 year	13 (5.7)	38 (16.7)	
	Total	47 (20.6)	181 (79.4)	0.407
	Gender			
	Male	26 (11.4)	91 (39.9)	
	Female	21 (9.2)	90 (39.5)	
	Total	47 (20.6)	181 (79.4)	0.624

Table 2. Comprehensive analysis of infection prevalence by age and gender

Variable	Infection		p-value
	Positive (%)	Negative (%)	
Age			
< 1 year	11 (1.8)	74 (12.2)	
1- 2 year	22 (3.6)	103 (17.0)	
2-3 year	31 (5.1)	99 (16.3)	
3-4 year	36 (5.9)	97 (16.0)	
> 4 year	34 (5.6)	99 (16.3)	
Total	134 (22.1)	472 (77.9)	0.070
Gender			
Male	75 (12.4)	225 (37.1)	
Female	59 (9.7)	247 (40.8)	
total	134 (22.1)	472 (77.9)	**0.053**

However, the differences in infestation rates across age groups are not statistically significant (p-value = 0.133). Male sheep have a slightly higher infestation rate (12.4% positive) compared to females (10.5% positive). Nonetheless, this difference is not statistically significant (p-value = 0.414). While the data suggest a trend where older sheep are more likely to be infested, the lack of statistical significance implies that age and gender alone may not be strong predictors of infestation in sheep. In goats, a gradual increase in infestation rates is observed with age, peaking at 3-4 years (6.0% positive). However, the differences across age groups are not statistically significant (p-value = 0.710). Similar to sheep, male goats show a higher infestation rate (13.7% positive) compared to females (9.5% positive). Despite this, the p-value (0.145) indicates that the difference is not statistically significant. The results suggest that while older goats and males may be more prone to infestation, these factors alone do not significantly influence the likelihood of infestation. Cattle display a consistent pattern of infestation, with the highest rates observed in animals older than 4 years (5.7% positive) and the lowest in those under 1 year (1.8% positive). Again, these differences are not statistically significant (p-value = 0.407). Male cattle have a slightly higher infestation rate (11.4% positive) than females (9.2% positive), but this difference is not significant (p-value = 0.624). Similar to sheep and goats, age and gender do not appear to be significant predictors of infestation in cattle, suggesting that other factors may play a more crucial role (Table 1-2).

Table 3. Prevalence of infection in analyzed muscles

Tissue	Number	Infection		Severity			P- Value	
		Positive (%)	Negative (%)	Mild	Moderate	Severe	Within Age	Within Gender
Esophagus	606	127 (21.0)	479 (79.0)	63 (10.4)	27 (4.4)	37 (6.1)	0.095	0.110
Skeletal Muscle	606	81 (13.4)	525 (86.6)	31 (5.1)	17 (2.8)	33 (5.4)	0.290	0.055
Diaphragm	606	105 (17.3)	501 (82.7)	45 (7.4)	24 (3.9)	36 (5.9)	0.008	0.133
Tongue	606	65 (10.7)	541 (89.3)	25 (4.1)	14 (2.3)	26 (4.3)	0.150	0.228
Heart	606	60 (9.9)	546 (90.1)	25 (4.1)	11 (1.8)	24 (3.9)	0.364	0.443

Table 4. Overall infection prevalence by macroscopic and digestive methods

Species	Infection		Inspection		
	Positive (%)	Negative (%)	Macrocyst (%)	Microcyst (%)	P-Value
Sheep	48 (22.9)	162 (77.1)	21 (10.0)	46 (21.9)	0.001
Goats	39 (23.2)	129 (76.8)	16 (9.5)	39 (23.2)	0.004
Cattle	47 (20.6)	181 (79.4)	25 (11.0)	47 (20.6)	0.008

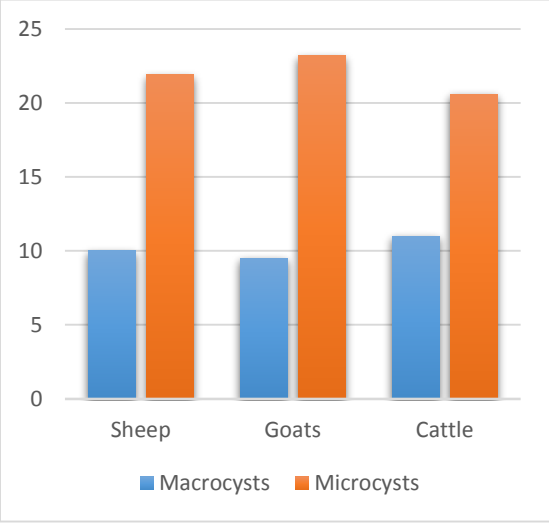

Fig 1. OverralliInfection prevalence by macroscopic and digestive methods

The esophagus has the highest infestation rate (21.0% positive). Severity levels vary, with mild, moderate, and severe infestations reported. The differences in severity by age and gender are not statistically significant (p-value = 0.095 and 0.110, respectively). Infestation in skeletal muscle is lower (13.4% positive), with no significant difference in severity levels (p-value = 0.290 for age, 0.055 for gender). The diaphragm shows a significant infestation rate (17.3% positive) and a statistically significant difference in severity levels by age (p-value = 0.008). This suggests that age may influence the severity of infestation in the diaphragm. Both the tongue (10.7% positive) and heart (9.9% positive) have lower infestation rates. There is no significant difference in severity by age or gender (p-values

> 0.05). The data highlight that certain tissues, like the esophagus and diaphragm, are more prone to infestation. The significant findings related to the diaphragm suggest that age might be an influencing factor for the severity of infestations in this tissue (Table 3).

Sheep show a significant difference between microcyst (21.9%) and macrocyst (10.0%) infestations, with a statistically significant p-value (0.001). This indicates that microcysts are more commonly observed during inspections. Goats also display a higher rate of microcyst (23.2%) compared to macrocyst (9.5%) infestations, with a significant p-value (0.004). Cattle follow a similar pattern, with microcyst (20.6%) being more prevalent than macrocyst (11.0%), and the difference is statistically significant (p-value = 0.008) (Table 4, Figure 1).

Discussion

Rasht city, with its pastures and numerous villages, is one of the most significant livestock regions in northern Iran. It is known for its high population of livestock. Sarcocystis, an intracellular parasite with global distribution, is prevalent among domestic ruminants and, in some species, humans. This parasitic infection is significant both in terms of public health and economic impact, causing millions of dollars in losses annually due to the condemnation of infected carcasses [25]. This study reveals a statistically significant difference between the results of macroscopic examination and digestive methods ($p < 0.05$). Macroscopic examination identified positive cases in goats (9.5%), in sheep (10.0%), and (11.0) in cattle. However, microscopic examination showed a much higher rate of infection, reporting 23.2% in goats, and 21.9% in sheep and 20.6% in cattle. The high prevalence of infection can be attributed to the abundance of stray dogs in farms, the practice of hand-feeding livestock, and storing fodder in barns, which protects the parasite from harsh environmental conditions. Goats' more selective feeding habits compared to cattle and sheep result in lower infection rates among goats. This finding highlights the insufficiency of traditional slaughterhouse inspection methods, such as ocular inspection, and underscores the utility and applicability of digestive methods for detecting infected samples. Jacob et al. (1960) also demonstrated that digestive techniques are effective in Sarcocystis investigations, as they found no macroscopic cysts, whereas 26.66% of samples were positive using digestive methods [13]. High rates of Sarcocystis infection have also been reported in neighboring countries. Latif et al. reported macroscopic infection rates of 0.2% in cattle and 4.1% in sheep in Baghdad, but digestive methods revealed 97.8% and 97% infection rates, respectively [17]. Ozturk in Turkey found 90% of studied sheep had microcysts [24]. This study does not reveal a statistically significant difference in infection rates among the different animal species. The prevalence of Sarcocystis infection was found to be 22.9% in sheep, 23.2% in goats, and 20.6% in cattle. These findings suggest that the level of environmental contamination is relatively consistent across these species, indicating that no single type of livestock is markedly safer from Sarcocystis infection than the others ($P>0.05$). Consequently, goat meat cannot be considered significantly safer in terms of Sarcocystis infection when compared to sheep and cattle meat. This underlines the importance of maintaining rigorous inspection and control measures across all types of livestock to ensure food safety. According to Zhou (1992), the prevalence of infection in domestic animals and humans in northern China was also 100% [31].

Based on the findings of this research, out of the 168 goats studied, 81 were male and 87 were female. Macroscopic examination identified 11 positive cases with a 6.5% infection rate in males and 9 positive cases with a 4.5% infection rate in females. Digestive method revealed 21 positive cases with a 12.5% infection rate in males and 18 positive cases with a 10.7% infection rate in females, which was not statistically significant ($P>0.05$). A statistically significant correlation was observed in the macroscopic examination and digestive method conducted on sheep ($P<0.05$). Specifically, 21 positive cases with 10% infection were identified as macrocysts, while 46 positive cases with 21.9% infection were reported. The

analysis did not reveal a statistically significant correlation between infection rates and the gender of the sheep. In the macroscopic examination, 12 positive cases (5.7%) were identified in male sheep, while 9 positive cases (4.3%) were found in female sheep. Similarly, the digestive method showed an infection rate of 12.4% in male sheep, with 26 positive cases, and a 10.5% infection rate in female sheep, with 22 positive cases (P>0.05). Cattle follow a similar pattern, with microcyst (20.6%) being more prevalent than macrocyst (11.0%), and the difference is statistically significant (P<0.05), indicating no relationship between sarcocyst infection rates and the sex of the animals (goats, sheep, and cattle) (p>0.05). In a study by Karegar Jahromi et al. in 2012, 9.8% of males and 8.7% of females had macrocysts, and all samples tested positive for microcysts, indicating no significant correlation between animal gender and infection rates, which aligns with our findings. Similar results have been reported in Kerman, Shiraz, Tabriz, and Baghdad [22, 4, 17, 28]. The findings of this study do not reveal a statistically significant relationship between infection prevalence and age. However, there is a notable trend of increasing infection rates with advancing age, with the highest prevalence observed in animals aged 3 to 4 years and those older than 4 years (P>0.05). This suggests that livestock are susceptible to Sarcocystis infection regardless of age, but the probability of encountering the infection appears to rise as the animals get older. These results underscore the importance of monitoring and managing the risk of Sarcocystis infection across all age groups, with particular attention to older animals, who may face a higher likelihood of infection due to prolonged exposure. In Australia, a study of 714 cattle showed that infection rates increase with age, reaching up to 92% in older males [27]. Carvalho in Lisbon also reported a direct correlation between age and infection rates, with infection varying from 0% in calves to 92% in older cattle [3]. Sarcocystosis, caused by various species of Sarcocystis, is a globally prevalent protozoan infection in various animal species and humans, affecting both striated and occasionally smooth muscles [19]. This study examined the muscles of the tongue, esophagus, heart, diaphragm, and skeletal muscles, identifying these organs as the most common sites of sarcocyst infection in cattle, sheep, and goats. In this study, no significant statistical relationship was found between infection rates and different muscles due to the absence of macrocysts (P>0.05), with esophagus and the diaphragm showing the highest infection rates and the heart the lowest. Various species of this protozoan form cysts of different sizes in animal muscle fibers, with some being visible to the naked eye and many others, similar to Toxoplasma tissue cysts, being microscopic. Thus, they often go undetected during visual inspections at slaughterhouses. Despite this, the highest incidence of Sarcocystis infection is found in the diaphragm muscle and subsequently in the esophagus during visual inspection. According to Fayer et al., even with this method, 100% infection rates in cattle and sheep were observed [7], and in another study, they also reported 100% infection rates in many animals, including cattle and sheep [9].

In the study conducted by Arshad et al. in Tabriz, the digestion method was identified as the most sensitive method for the accurate detection of Sarcocystis infection in sheep. Using macroscopic inspection, the highest incidence of Sarcocystis infection was observed in the diaphragm, with most cysts appearing as large, rice-grain-sized cysts. In the microscopic tissue spread methods, both with and without staining, the diaphragm was also found to be the most infected tissue [1]. Similarly, in a study conducted by Shakerforush et al. in 2004 on the incidence of Sarcocystis infection in cattle carcasses at the Isfahan slaughterhouse, no macroscopic cysts of the parasite were observed in any of the 250 cattle examined. However, 94.8% of the tested cattle were infected with microscopic Sarcocystis cysts. The distribution of microcyst infection in various muscles was as follows: heart muscle (84.4%), esophagus (53.6%), diaphragm (37.2%), and tongue (29.6%) [28].

Conclusion

This study clearly demonstrates the widespread prevalence of Sarcocystis infection among

various livestock, including goats, sheep, and cattle in Rasht city. The results from different testing methods reveal significant differences in infection detection rates. While macroscopic examination identifies fewer infection cases, the digestive method proves significantly more effective in detecting microscopic infections. These findings underscore the importance of using more precise and sensitive methods for detecting Sarcocystis infections, as traditional methods like visual inspection are insufficient for comprehensive detection. Additionally, the study highlights that goats have a lower infection rate compared to sheep and cattle, likely due to their more selective feeding habits.

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Not applicable

Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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