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Sarcosporidiosis of the Ovine Esophagus: Frequency and Histopathological Identification of the Species Involved in Tiaret, Algeria

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ABSTRACT

Sarcosporidiosis is a common parasitic disease due to protozoa of the Sarcocystis genera. This study, conducted in the Tiaret region of Algeria from September 2018 to April 2019, aimed to determine the global frequency of macrocyst esophageal localization, identify risk factors such as sex and age, and conduct a histopathological study to identify the involved species. The infection's global frequency was 22.77%, the majority of which were sheep over 3 years of age. The females were the most infested compared to the males at 25.13% against 12.20%; the degree of infection was important at 9.31 cysts per infested case. The histopathological study revealed that *S. gigantea*, whose definitive host is cat, was the causative species of macroscopic Sarcosporidiosis. However, another species with a microscopic character has been revealed during this study; it's *S. tenella*. The association between the two species was 31%. To conclude, more control is needed in order to reduce this infection in the study region through the destruction of seizures at the slaughterhouse level and hygiene at the farm level.

Keywords: Esophagus, Sarcosporidiosis, Sheep, Slaughterhouse, Tiaret.

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INTRODUCTION

Sarcocystosis is a protozoal disease affecting a wide range of animals (**Pagano** et al., 2020), caused by Sarcocystis spp. (Protozoa: Apicomplexa). This cystogenic parasite has an obligatory two-host cyst cycle (**Máca**, 2020). Infections are recognized to occur in all parts of the world, and farm animals are intermediate hosts for a number of species. Sheep are the intermediate host for four species: two microcyst species (S. tenella [syn. S. ovicanis] and S. arieticanis) and two macrocyst species (S. gigantea [syn. S. ovifelis] and S. medusiformis) (**Bittencourt** et al., 2016).

The consequences of sarcocystosis depending the host (definitive. different on intermediate, or accidental) and the stage of the disease. In intermediate hosts such as sheep, the infection is commonly asymptomatic, and the presence of muscular cysts is considered an incidental finding occurring in virtually all sheep at the time of slaughter. However, some species of Sarcocystis, particularly those with a canid as the definitive host, may cause clinical signs including fever, inappetence, and

reduced productivity (**Pagano** *et al.*, **2020**). Microcysts are formed mainly in the heart, esophagus, tongue, and diaphragm muscles, but they can also be found in several skeletal muscles and in the central nervous system. In turn, macrocysts generally appear in the esophagus and diaphragm muscles of sheep (**Dubey and Lindsay**, **2006**; **Minuzzi** *et al.*, **2019**).

These macroscopic cysts are classified as carcass lesions in many countries, and infected carcasses may be rejected from export or condemned for human consumption (O'donoghue and Ford, 1986). Sarcocytosis in definitive hosts is most often asymptomatic except for self-limiting mild diarrhea (Stojecki et al., 2012), but in intermediate hosts, the acute form of sarcocystosis can cause central nervous system signs, abortion, premature delivery and even death of the infected animals. However, the chronic form leads to a decrease in body weight and a reduction in wool quality and milk yield (Shahraki et al., 2018). The intermediate host (herbivors or omnivors) becomes infected by the ingestion of sporocysts (Shahraki et al., 2018) and there,

merogony and cyst formation (a sexual stage) take place (**Dubey and Lindsay**, 2006).

On the other hand, the definitive one (carnivores) becomes infected by ingestion of muscles containing bradyzoites, which are the mature *Sarcocystis spp.* cyst (**Fayer, 2004**) and inside this host, the sexual stage (gametogony and sporogony) takes place (**Dubey and Lindsay, 2006**). In general, the *Sarcocystis* species that are transmitted via canids or primates are more pathogenic than those transmitted by felids (**Abdallah** *et al.*, **2021**).

This study aimed to investigate the occurrence of macroscopic Sarcosporidiosis and the histological identification of *Sarcocystis* species in the infected esophagus of sheep slaughtered in the municipal abattoir of Tiaret, Algeria.

MATERIALS AND METHODS

Study area

The present study was conducted in the abattoir of Tiaret. The region is situated in the high plateau of Algeria, a semi-arid area characterized by a cold humid winter and a hot dry summer (**kouidri** *et al.*, **2017**). The laboratory work was done at the Veterinary Sciences Institute of Ibn Khaldoun University of Tiaret.

Sample collection

In this study, 224 tissue samples from esophagus of females (183) and males (41) sheep of different ages were randomly collected from Tiaret municipal slaughterhouse during the months from September 2018 to April 2019.

Macroscopic study

The entire length of each esophagus was thoroughly examined by close visual inspection for infections by macroscopic cysts, and all cysts found were collected for morphological and histological studies.

Microscopic study

The positive cases were directed to the parasitology laboratory of the Veterinary Sciences Institute of Tiaret. We made a crushing between the glass slide and coverslip of a cyst already removed from the esophagus, then we proceeded to stain with methylene blue or eosin to demonstrate banana-like bodies or Rainey's corpuscles; the observation was made under an optical microscope at 10 and 40 magnifications. The number of cysts in each infected esophagus was also counted.

Histopathological study

Formalin-fixed esophageal samples were routinely processed through an automatic tissue processor Leica TP1020 at the Histopathology

Laboratory of the Veterinary Sciences Institute, University of Tiaret. Then, the tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) according to the technique described by **Suvarna and Layton (2018).** Photomicrographs of selected lesions were taken using an optical microscope (Zeiss) equipped with a camera (AwioCamEPc 5s). Stained sections were observed for histological identification of *Sarcocystis* species.

RESULTS

In this study, sarcocysts were detected macroscopically as whitish, rice-grain-like shaped in the muscular layer of the sheep esophagus. Among the 224 oesophagi inspected, 51 were found to be positive, which represents a rate of 22.77% (**Table 1**).

Table 1: Percentage (%) of positive samples presenting Sarcocystis bradyzoites in the esophagus of male and female sheep by the digestion technique.

	Number of Samples		Esophagus samples with positive Sarcocystis bradyzoites	
			No	%
Esophagus	Female	183	46	25.13
	Male	41	5	12.20
	Total	224	51	22.77

One to several sarcocysts have been identified along the esophagus (**Fig. 1**). Macroscopic counting of the sarcosporidian cysts has revealed a highly variable frequency (1 to 45) among examined esophagus samples (**Fig. 2**). The obtained results showed a highly significant occurrence of esophageal positive samples in females (25.13%) compared to males (12.20%) (**Table 1**).



Fig. 1: Esophagus of sheep presenting multiple oval-shaped macroscopic sarcocysts.

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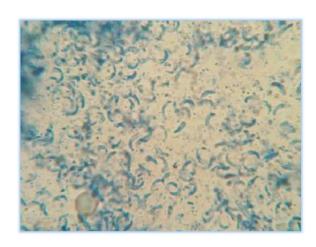


Fig. 2: Light microscope of bradyzoites stained with Methylene blue (100 x).

As shown in **Table 2**, a very high occurrence of positive samples with Sarcocystis bradyzoites was observed in female and male sheep over 3 years of age, with 53.97% and 33%, respectively. Sheep aged between 1 and 3 years have recorded almost similar occurrences in males (19%) and females (17.19%); however, sheep under one year of age showed a very low occurrence of sarcocystis bradyzoites in females (1.79%) and null in males.

Table 2. Sex and age distribution (%) of positive samples with Sarcocystis bradyzoites in the esophagus of sheep by the digestion technique, (n=224).

Age		Esophagus samples			
(year)		Examined	Infected	%	
< 1	Male	17	0	0	
	Female	56	1	1.79	
1 to 3	Male	21	4	19	
	Female	64	11	17.19	
> 3	Male	3	1	33	
	Female	63	34	53.97	

The histologic analysis of esophagus sections in sheep revealed sarcocysts with variable sizes and shapes (round or elliptical), associated with the angle of tissue sectioning (**Fig. 3,4 and 5**). Histological examination of ositive cases allowed the identification of two sarcocystis species, namely, *S. gigantea* and *S. tenella*, with 46% and 23% occurrence, respectively (**Table 3**).

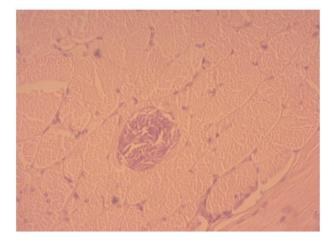


Fig. 3: Cross section through an esophagus muscularis layer with a *Sarcocyst*, 100x, H&E.

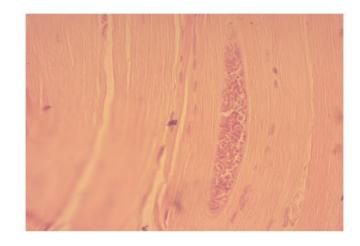


Fig. 4: Transversal section of sarcocyst through an esophagus muscularis layer, 100x, H&E.

Table 3. Comparative occurrence of Sarcocystis species in positive esophagus samples by histopathology(n=13).

	Sarcocystis species			
	S. gigantea	S. tenella	Association	
Occurrence	46% (6/13)	23% (3/13)	31% (4/13)	

However, mixed infection was observed in 31% of total positive cases. Sarcocystis tenella is protrusions: thick-walled with villar however, thin-walled Sarcocystis gigantea is and has cauliflower-like villar protrusions (Figs. 6 and 7). In some cases, a mild, multifocal lymphoplasmacytic inflammatory infiltrate of endomysium was observed around parasitized and nonparasitized muscle fibers. Remarkably, no eosinophils were observed in the examined positive samples.

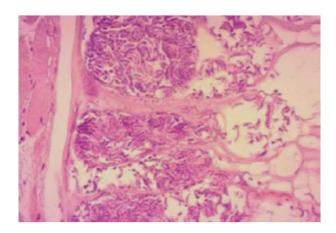


Fig. 5: Esophagus of sheep, cross section of a sarcosporidian cyst. 100x, H&E.

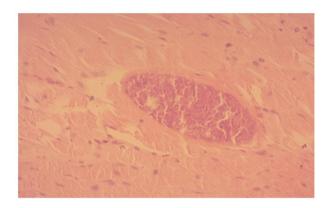


Fig. 6: A thick walled, radially striated sarcocyst containing myriad of banana-shaped bradyzoitesthrough the muscular layer of sheep esophagus, (*S. tenella*)100x, H&E.

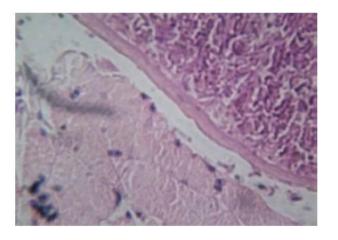


Fig. 7: A thick walled sarcocyst containing myriad of banana-shaped bradyzoites through the muscular layer of sheep esophagus, *S.gigantea* 400x, H&E.

DISCUSSION

Slaughterhouse inspection is one of the best means of confirming certain parasites whose pathology is discreet (**Blaise**, 2001). The results obtained during the present study allowed us to draw the following conclusions concerning sarcosporidiosis with macroscopic cysts located on the esophagi of sheep

slaughtered. According to the present study, the frequency of ovine macroscopic sarcosporidiosis is 22.77% (51/224). This value is close to that reported by **Pipia** *et al.*, (2016) in Sardinia, Italy, which was 23.3%. However, **Dahmani** *et al.*, (2017) recorded a lower frequency of 1.03% in the El Harrach region (Algeria). **Nedjari** (2002) in the region of Algiers did not record any cases, as did the study carried out by **Pedro** *et al.*, (2016) in Brazil.

According to the results of our study, females are more affected by sarcosporidiosis compared to males, with a rate of 25.13% compared to 12.20%. According to **Rahmani** *et al.*, (2012), they are more affected because they live longer than males. In addition, the stress of pregnancy, parturition and lactation can also promote the development of the parasite (**Iqbal** *et al.*, 2012).

The age category most affected, according to our study, was that of those over 3 years old, with 53.97% among females and 33% among males. This result can be justified by the more or less slow duration of cyst development. As a result, animals slaughtered at an early age may not present the disease, as was reported by **Nourani** et al., (2010) in Iran. This study highlighted a significant degree of infection with a total of 465 cysts, therefore an average of 9.31 and a standard deviation of 8.99, which is significantly higher than the result of **Fassi-Fehri** et al., (1978) in Morocco, who reported a mean of 5.9 and a standard deviation of 4.1.

Regarding the histopathological study, *S. gigantea* was the most frequent Sarcocystis species, with a rate of 46%, which is significantly higher than the value described by **Dahmani** *et al.*, (2017), which was 5.67%. However, reading the slides revealed another cyst with a thick, radially striated wall, which corresponds to a microcyst called *S. tenella*, with a percentage of 31%. Nedjari (2002) recorded a rate of 60.63%, and **Dahmani** *et al.*, (2017) reported 43.88%. The combination of the two species represented 23% of cases. Dahmani *et al.*, (2017) recorded a percentage of 20%; **Mounika** *et al.*, (2017) in India reported a lower rate of 0.04%.

CONCLUSION

At the end of our study, we can conclude that ovine sarcosporidiosis is a common parasitosis, with an overall frequency of 22.77%. Sheep aged over 3 years were the most infected. As for the species involved, the histopathological study showed that *S. gigantea* was the species identified as responsible for the sarcosporidian macrocysts. However, the microcysts were caused by *S. tenella*. In light of the results obtained, we propose more control in order to reduce, or if possible, eradicate, this parasitosis in the study region (Tiaret); ensure rigorous inspection and

total seizure of infected organs; prevent contamination of sheep's feed or water by carnivore feces, require regular deworming of dogs and cats while collecting and destroying feces; and avoid giving raw or undercooked meat to dogs or cats.

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