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MACROSCOPIC PARASITIC LESIONS OF SHEEP MEAT AT TWO SLAUGHTERHOUSES IN THE NORTH OF ALGERIA

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ABSTRACT

A total of 10,696 randomly selected sheep have been collected in two slaughterhouses in the north of Algeria (El Harrach and Boufarik) to determine the prevalence of muscular cysticercosis and macroscopic cysts of sarcosporidiosis, to find out the association between prevalence and potential risk factors, as well as to assess the distribution of these parasites in the surface's muscles of slaughtered sheep. All of the slaughtered sheep carcasses were visually and carefully inspected. Cysticercosis and sarcosporidiosis were found in 220 (2.06 %) and 76 (0.7 %) sheep, respectively. For both diseases, the prevalence was significantly higher in females than males. The prevalence of *Cysticercus ovis* increased with age, and the difference was statistically significant ($P < 0.0001$), while all infected animals were old (over 5 years old) for sarcosporidiosis. For *C. ovis*, it didn't have significant difference between the seasons, however, all sheep were infected in the spring for sarcosporidiosis. All the detected cysts of *C. ovis* were non-viable, and were more frequently detected in the heart (51.82 %), followed by the diaphragm (30.77 %) and the oesophagus (17.41 %). This anatomical distribution of *C. ovis* cysts showed a significant variation ($P < 0.001$) in different predilection sites. Whereas, for sarcosporidiosis, the species involved was identified as *S. gigantea* by histology and were distributed in the organs as follows: the skeletal muscles (38.15 %), the oesophagus (31.57 %) and the diaphragm (30.26 %); no significant difference were noted between the 3 sites. The present study has revealed that these parasites are present in Algeria. Appropriate control measures need to be introduced to eradicate these parasites in sheep.

Key words: muscular cysticercosis; sarcosporidiosis; sheep; slaughterhouses

INTRODUCTION

The infection of meat with macroscopic parasitic cysts can involve both protozoan and helminth species. Cysticercosis and sarcosporidiosis constitute the major part.

Cysticercosis in livestock and wild animals is caused by the larval stages (metacestodes) of cestodes (taeniosis), the tape worm of humans, dogs and wild canids. Muscle cysticercoses in sheep are caused by *Tænia ovis* (the adult stage of *Cysticercus ovis*), of which adults develop in the intestines of dogs and wild canids [29]. Infections with the

larval stage of some species of *Taenia* are of veterinary importance because they cause economic losses. These losses are associated with total condemnation of infected offal or carcasses with generalized infestation and downgrading of carcasses which are subjected to refrigeration, in addition to the cost of refrigeration and transport [24]. Like beef and pork cysticercosis, ovine cysticercosis due to *Cysticercus ovis* is of special interest in meat inspection because it affects the musculature, that part of the animal which is at once the most valuable for food purposes and the most difficult to inspect thoroughly.

Sarcocystis is one of the most prevalent protozoan parasites in the striated muscles of livestock such as cattle, sheep and goat slaughtered for human consumption [5]. Sheep are intermediate hosts of five *Sarcocystis* species: *Sarcocystis tenella* (synonym *Sarcocystis ovicanis*), *Sarcocystis gigantea* (synonym *Sarcocystis ovifelis*), *Sarcocystis arieticanis* and *Sarcocystis medusiformis*. *S. gigantea* and *S. medusiformis* are transmitted by felids and are non-pathogenic. *S. tenella* and *S. arieticanis* are transmitted by canids and are pathogenic [13]. Another species, *Sarcocystis mihoensis* has been recently reported to occur in sheep [31]. The losses caused by discrimination of the carcasses with macroscopic cysts during meat inspection represented a serious economic problem. Despite it all, sarcosporidiosis in Algeria is underestimated, because the inspection of the carcasses for sarcosporidiosis is not obligatory.

In Algeria, unlike cattle, cysticercosis and sarcosporidiosis in sheep are not taken into consideration because they are not zoonotic and no legislation has been put in place concerning these diseases. Indeed, there are limited reports concerning these parasitic diseases in slaughtered animals. Considering the importance of these pathologies, and the lack of epidemiological information concerning these diseases in Algeria, the present work was conducted to determine the prevalence of muscular cysicercosis and macroscopic cysts of sarcosporidiosis from sheep in two slaughterhouses (El Harrach and Boufarik) in the north of Algeria.

MATERIALS AND METHODS

Climatic conditions in northern Algeria

Along the country's northern Mediterranean coast, the climate is typically Mediterranean with hot, dry summers, but mild, rainy winters. Rainfall is between 600 mm and 800 mm. Most of the annual precipitation occurs between October and April. The temperatures are rather uniform: the average daily temperature (maximum and minimum) is around 11 °C – 12 °C in January, the coldest month of the year, while the average daily temperature (maximum and minimum) is around 25 °C – 26 °C in July – August, the hottest months of the year. Summers are long and sunny, with rather humid air on the coast, but also with sea breezes.

Inspection of sheep carcasses for macroscopic cysts and sampling method

10,696 sheep carcasses were inspected over a two-year period, from January 2017 to December 2018 in two abattoirs in the north of Algeria (7,856 sheep in El Harrach and 2,840 in Boufarik) during our study for *Cysticercus* spp. and macroscopic species of *Sarcocystis* spp. The sheep were native breeds and they originated from different sub-districts within the municipality and their environs. The animals inspected, were of different sex and age. A few females were sampled only, because the females are kept for breeding purposes, age was estimated and the animals were divided into three age groups: ≤1 year, 1.5—3 years and 3—5 years. All of the slaughtered sheep carcasses were carefully inspected, with particular attention to the muscles (skeletal muscle, diaphragm, oesophagus, heart). The tongue and the masseters were not examined, because the head of the animals is separated from the carcass and delivered to trade without being examined. Macroscopic lesions namely translucent oval containing clear fluid, sometimes bloody, with a white point (protoscolex) or degenerate cyst were considered to be *C. ovis*. While, macroscopic lesions of sarcosporidiosis in the form of whitish ovoid to fusiform cysts with a few millimetres long were packaged in an identified plastic bag, and taken to the Laboratory of Parasitology and Mycology in the Superior National Veterinary School, Algiers for confirmation of the species in question by direct examination and the histology technique.

Identification of macroscopic sarcosporidiosis cysts

Direct examination

Identification of the contents of macroscopic *Sarcocystis* cysts by direct examination consists of spreading the contents

on a slide followed by MGG (May-Grünwald Giemsa) staining according to the method cited by Bussiéras and Chermette [8]. The slides were observed under an optical microscope 400× magnification to look for bradyzoites (RAINEY's corpuscle) of *Sarcocystis* spp.

Histological technique

All samples were prepared in the Laboratory of Anatomy Pathological at the Superior National Veterinary School, Algiers. The fixed tissue samples were cut into 0.5cm thick sections, dehydrated with serial dilutions of ethanol and xylene, processed into paraffin, sectioned to 4 to 5 microns, stained with haematoxylin and eosin (H&E), and examined with the light microscope at magnification (100×, 400×, 1000×). The wall morphology of cysts in the muscle sections were examined by the light microscope with immersion oil (1000×) for the identification of the species.

Statistical analysis

For statistical analysis, we used the software program Microsoft Excel 2010. The comparison of the distribution of different populations were analysed by using Chi-Square test with the level of significance $P < 0.05$.

RESULTS

Of the 10,696 carcasses examined, ovine cysticercosis was found in 220 (2.06%) carcasses, while 76 (0.7%) sheep were found to be infected with macroscopic cysts of *Sarcocystis* spp.

Prevalence according to the predilection sites

The rate according to the predilection sites is represented in Table 1.

All muscular cysticercosis cysts found in sheep slaughtered were dry, in the form of hard whitish vesicles,

Table 1. Prevalence of cysticercosis and sarcosporidiosis according to the predilection sites

Organs	Organs infected with muscular cysticercosis of *C. ovis*		Organs	Organs infected with macrocysts of *Sarcocystis* spp.	
	N	%		N	%
Heart	128	51.82	**Skeletal muscles**	29	38.15
Diaphragm	76	30.77	**Diaphragm**	23	30.26
Oesophagus	43	17.41	**Oesophagus**	24	31.57
Total	**247**	**100**	**Total**	**76**	**100**

Table 2. Prevalence of cysticercosis and sarcosporidiosis according to risk factors

Factors	Category	Number of sheep examined	Sheep with muscular cysticercosis of *C. ovis*		Degree of significance and p value	Sheep with macrocysts of *Sarcocystis* spp.		Degree of significance and p value
			N	%		N	%	
Gender	Male	10641	200	1.88	P < 0.0001	26	0.24	P < 0.01
	Female	55	20	36.36		50	90.90	
Age Year (s)	≤ 1	2267	5	0.22	P < 0.0001	0	0	
	[1.5—3]	5269	79	1.50		0	0	
	[3—5]	3160	136	4.30		76	2.40	
Season	Winter	2280	56	2.46	P > 0.05	0	0	
	Spring	1439	45	3.13		76	5.28	
	Summer	3655	71	1.94		0	0	
	Autumn	3322	48	1.44		0	0	

pearlescent sometimes yellowish, about 1 cm in diameter on average, which could correspond to *C. ovis*. These cysts had a tendency to be located more in the heart (Fig. 3), followed by the diaphragm. However, a low percentage were found in the oesophagus and this difference between infections rate of heart and other organs was highly significant ($P<0.001$).

Sarcosporidiosis lesions were observed on the skeletal muscles (intercostal muscle and thigh), followed by the oesophagus (Fig. 4), then the diaphragm (Fig. 5). These organs were infested with several whitish fusiform to ovoid cysts 2 mm to 1.5 cm long. No significant differences were observed between the 3 preferred sites.

Prevalence according to risk factors

The prevalence of ovine cysticercosis and sarcosporidiosis in relation to the season, the age and sex of sheep is shown in Table 2.

Identification of macroscopic cysts of *Sarcocystis* spp.

Microscopic examination of the contents of the cysts (Fig. 1) allowed the observation of bradyzoites (banana-shaped structures) in all the samples examined.

Histological technique identified macrocysts with the thin wall and cauliflower-like protrusions surrounded by the secondary cyst wall corresponding to the description of *S. gigantea* (*S. ovifelis*), as shown in Fig. 2.

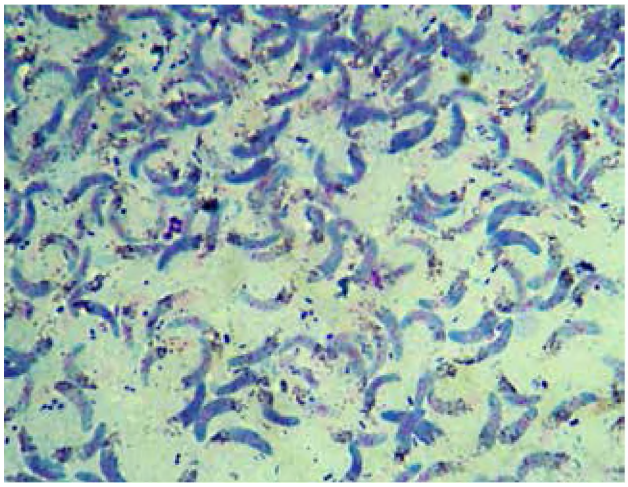

Fig. 1. *Sarcocystis* bradyzoite observed in samples of sheep oesophagus after MGG staining (400×)

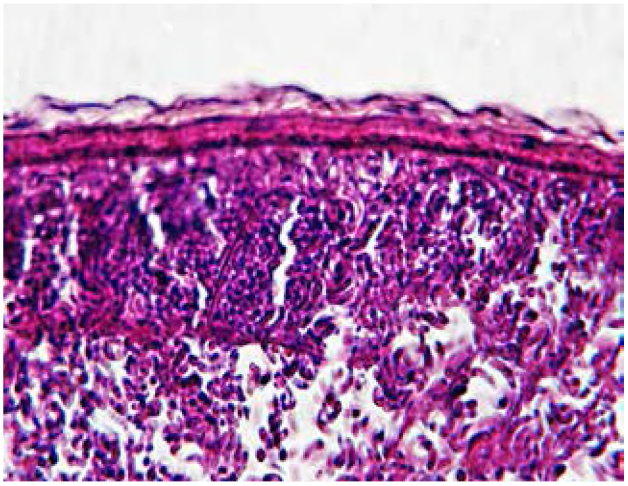

Fig. 2. *S. gigantea* in oesophagus with secondary cyst wall and cauliflower protrusions (H and E, 1000×)

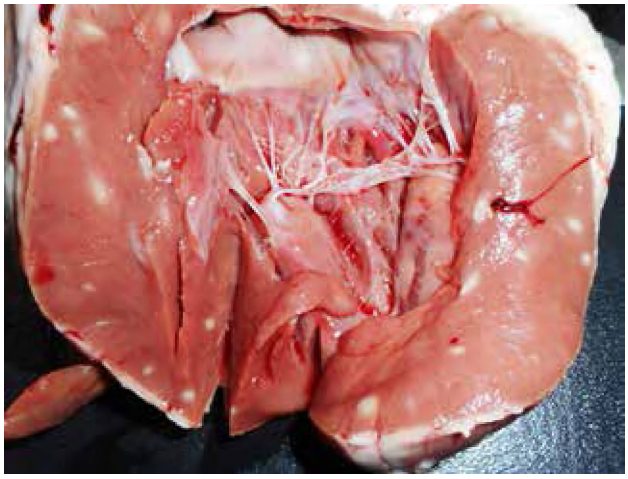

Fig. 3. Degenerated *C. ovis* cysts in the heart of sheep

Fig. 4. Ovoid macroscopic cysts of *Sarcocystis* spp. in oesophagus of sheep

Fig. 5. Fusiform macroscopic cysts of *Sarcocystis* spp. in diaphragm of sheep

DISCUSSION

Overall prevalence

In our study the prevalence of muscular cysticercosis was found to be 2.06%. Similar findings have been reported in Benin (3.44%) [4], in Bulgaria (2.4 and 3.1%) in 1980 and 1981, respectively [18], in Southern Australia (2.39%) [35] and in Nigeria (1%) [10]. While higher prevalence compared to current results was reported from other countries: 7% in England [14] and 11.14% in the USA [21].

Christodoulopoulos et al. [9] noted that the infection is influenced by several factors, such as, the improper disposal of dead animals, the access of farm dogs to the offal of slaughtered sheep, the carelessness of farmers to treat farm dogs with anthelminthics, and the grazing of flocks in fields where stray dogs have free access.

The disease is often associated with a lack of hygiene and poor farming practices. Samuel and Zewde [32] concluded that the prevalence is influenced by the type and stocking density and husbandry practices. The role of dogs is important in the transmission of pathology. Eichenberger et al. [14] showed that the frequent use of fields by dog walkers represented a high risk for transmission of *T. ovis*.

In our study, all the cysts detected were non-viable. Broadbent [7] noted that 99.8% of the cysts detected were non-viable. Sheep probably have a good immune system that allows them to fight cysticercosis cysts. In effect, Euzeby [16] noted that this evolution of the lesions is due to the immune reaction of the host.

The reason for the low prevalence of muscular cysticercosis recorded in this study may be related to the low level of environmental contamination, also, our findings were based on surveys carried out on carcasses subjected to routine meat inspection procedures. This can possibly underestimate the incidences reported. According to Euzeby [15] in experienced meat inspectors could most likely miss out quite a number of viable cysticerci, which blend with the pinkish-red colour of the meat, or which are lodged in the intermuscular connective tissue, generally infiltrated with fat. Dorny et al. [11] reported that the sensitivity of visual inspection is considered low (<30%). However, it is believed that visual inspection of the meat can identify heavily infected carcasses, which also poses the greatest risk.

Macroscopic examination of sheep carcasses revealed the observation of macroscopic white cysts of *Sarcocystis* spp. with a prevalence of 0.7%. In Algeria, Nedjari [27] failed to confirm the presence of macroscopic cysts of sarcosporidiosis. In addition, from Australia, Hinaidy and Egger [20] revealed a prevalence of 2.4%, O'Donoghue and Ford [28] observed macroscopic cysts in 6.7% of the samples, but detected 2 types of species: *S. gigantea* (4.5 %) and *S. medusiformis* (3.1%). Furthermore, other studies revealed a high prevalence of infections, in Turkey, the prevalence varies from one study to another: 7.89% was recorded by Aldemir et al. [3], 17.5% by Aldemir [2] and 24.5% by Beyazit et al. [5], while in Jordan a rate of 11.3% was noted by Abo-Shehada [1]. On the other hand, Fassi-Fehri et al. [17] in Morocco and Vercruysse and VanMarck [34] in Senegal failed to observe macroscopic cysts.

In our study, the species identified was *S. gigantea* by histology. Differentiation between this species and other species of *Sarcocystis* has been made on the basis of cyst morphology and wall appearance [5, 12]. *S. gigantea* cysts are completely different. Protrusions are cauliflower-like,

reach a length of 4.5 µm. In addition to these primary cyst wall differences, this species has a secondary cyst wall, which is always lacking in *S. tenella*, *S. arieticanis*, and *S. medusiformis* [12].

Sheep infested with the cysts probably lived close by the cats which are the definitive hosts, and were infected by ingesting the oocysts from the cat faeces.

Prevalence according to the predilection sites

The present abattoir study revealed that among the organs accessible for detailed inspection, the heart, was the most affected by cysticercosis. This preferred predilection was similar with earlier findings reports by Bilan and Tassin [6], Jensen et al. [21], and Kebede [23].

Euzeby [16] noted that the elective muscular localizations were the heart, the masseter and pterygoid, the diaphragm and the tongue. From Kebede [23], the distribution of lesions on the various organs can be influenced by several factors such as muscle activity, age, and the geographic area.

For sarcosporidiosis, the skeletal muscles are the most infected with these cysts followed by the oesophagus and the diaphragm. In Iran, the species observed were *S. gigantea*, mainly in the oesophagus, *S. medusiformis*, mainly in the diaphragm [30]. In Turkey, the highest prevalence of *S. gigantea* was recorded in the oesophagus [5]. On the other hand, Meshkov and Kotomindev [25] found that macroscopic cysts were present mainly on the exterior and interior parts of carcasses (abdomen and thorax).

No inflammatory lesions were observed in the evaluated sheep. The same results were observed by Beyazit et al. [5] and Tinak [33]. However, Jensen et al. [22] reported that all carcasses were discriminated for eosinophilic myositis indicating that opened sarcocysts killed the myocyte-host by causing the granuloma formation.

Prevalence according to risk factors

Results from our study showed an increase in prevalence of ovine muscular cysticercosis with age confirming that a major source of infection for sheep is likely to be through the consumption of *Taenia* eggs from the environment. These results are similar to those of some previous investigations [4, 9, 35] indicating that age was an important factor for being positive as a measure of the cumulated life-time risk.

This study used the samples from abattoir where the majority of the slaughtered animals were male, and the only females less than 5 years old were slaughtered in accordance with Algerian regulations because females are preserved by breeders for reproduction. This may not reflect the reality of difference between gender in the occurrence of muscular cysticercosis.

In Benin, Attindehou and Salifou [4] noted that the infection was prevalent in all seasons with nevertheless high rates in the rainy seasons. In the USA, Jensen et al. [21] found that the prevalence was higher in the autumn than in the spring. The authors assumed that the lambs reached the infection from summer pastures contaminated by *T. ovis* eggs.

For sarcosporidiosis, the prevalence was significantly higher in females than males, while all infected animals were old (over 5 years old). The adult sheep are most often infected by the macrocysts [1]. For this reason, most infected sheep were old females over 5 years old, which is in accordance with the Algerian regulations stipulating slaughtering after that age. This may be due to the exposure time of sheep to the oocysts present in the faeces of the definitive host which is short for the young lambs. Or, to the prolonged development of these macroscopic cysts because species transmitted by felines require long periods of time to become infectious in intermediate hosts [19]. Munday [26] noted that of macroscopic sarcocysts in sheep was found in 0.6% of lambs, 8.8% in sheep (1 to 4 years old) and 66% in (≥4 year olds). However, Beyazit et al. [5] found cysts of *S. gigantea* in 3 sheep under 1 year, and suggested that macrocysts might develop in less than 1 year.

CONCLUSIONS

This study has revealed a non-negligible prevalence of ovine cysticercosis and sarcosporidiosis in the two slaughterhouses in the north of Algeria, suggesting a dispersion of *Sarcocystis* spp. oocysts and *T. ovis* eggs and parasite reservoir hosts in the environment. This study highlights the impact of these parasitic diseases in the study region, further studies are needed to determine the impact of these diseases in different regions in order to stimulate better efforts towards the control and possible eradication of these diseases.

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