

Seasonal variation of lungworm infestation of ewes at Sidi Bouzid slaughterhouse (Central Tunisia)

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Summary

Respiratory strongyloses are among the most important diseases affecting sheep reared in various farming systems in many countries. In Tunisia, farmers without any scientific background carry out the control of these parasites. A survey was performed in a slaughterhouse in Sidi Bouzid District (Central Tunisia) to assess the prevalence of sheep infestation by lungworms. The study lasted 12 months and involved 720 of the slaughtered ewes. Fecal samples from each ewe were collected and examined by Baermann's technique. Sixty-eight samples were infested with pulmonary nematode larvae ($9.4 \pm 2.1\%$). The population was dominated by *Protostrongylus rufescens* ($4.4 \pm 1.5\%$), followed by *Dictyocaulus filaria* ($2.6 \pm 1.2\%$), *Cystocaulus ocreatus* and *Neostongylus linearis* ($1.3 \pm 0.8\%$), and finally *Muelle-rius capillaris* ($0.3 \pm 0.4\%$), whereas mixed infestations concerned $0.4 \pm 0.5\%$ of samples. Lungworm infestation was observed in all age groups with no significant difference ($p = 0.64$). In addition there was no significant difference in infestation between fat-tailed Barbarine ($10.8 \pm 3.6\%$) and Queue fine de l'Ouest (Western Thin Tail) ($8.5 \pm 2.6\%$; $p = 0.31$) sheep breeds. The lowest prevalence was recorded in spring ($6.7 \pm 3.6\%$) and the highest in winter ($11.7 \pm 4.8\%$), but the difference between seasons was not significant ($p = 0.32$). This study concerning the activity dynamics of lungworms should be completed by a monitoring project to determine the economic importance of these infestations and provide a solid basis for the establishment of specific control programs against these parasites in Tunisia.

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■ INTRODUCTION

Sheep are one of the most important sources of meat in Tunisia. The sheep population has been estimated at 3.763 million head producing 111,000 tons of red meat per year (Tunisian Ministry of Agriculture, 2016). With a sheep population of 662,200 head, Sidi Bouzid District ranks first in Tunisian sheep production (Tunisian Ministry of Agriculture, 2016).

Despite its importance, several constraints contribute to a low productivity of the sheep sector, such as traditional and poor

management, a long feeding gap during the dry season, and the high prevalence of diseases (mainly viral, bacterial and parasitic). In several countries and production systems, lungworms are responsible for huge economic losses in meat and milk production and cause mortality in sheep, goats and cattle (Soulsby, 1982), especially because the lesions caused by the worms may be complicated by bacterial bronchopneumonia. The life cycle of these parasites is influenced by geographic and climatic factors (Panayotova-Pencheva, 2011) leading to specific regional dynamics (Phillips et al., 2010). Infestation of small ruminants occurs through ingestion of grass contaminated by infesting larvae or hosting the intermediate hosts of the larvae of parasites with indirect life cycle (Snyder et al., 2015). The infestation impact depends on the host susceptibility, the host-parasite interaction and the number of larvae ingested.

In Tunisia, several studies have been carried out on gastrointestinal nematodes (Lahmar et al., 1990; 2004; Akkari et al., 2011; 2012; 2013; Rouatbi et al., 2016), but as far as it could be ascertained, there has been no longitudinal survey regarding respiratory lungworms, except a few veterinary theses studying

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the histological effects of these parasites (Aloui, 2005; Temri, 2007; Ben Said, 2008). Lungworm importance still needs to be assessed. Therefore, the aim of this study was to investigate the seasonal variation of lungworm infestation in sheep in Sidi Bouzid region.

MATERIALS AND METHODS

Study area

Sidi Bouzid is located in the center of Tunisia (35° 02' N; 9° 29' E) (Figure 1). It covers 7405 square kilometers representing 5% of the total area of the country. The climate of Sidi Bouzid is Mediterranean continental: it is arid in the southern part of the district and semi-arid in its northern part. Winter is cold and dry (mean temperature of 11.8°C) whereas summer is hot (28.2°C). Mean annual rainfall is low (287 millimeters) with high interannual and interseasonal variations.

Animals and coprologic samples

Monthly visits were implemented between May 2014 and April 2015 at Sidi Bouzid regional slaughterhouse. Feces were collected from 720 female sheep (60 per month) belonging to two breeds, fat-tailed Barbarine (287) and Queue fine de l'Ouest (Western Thin Tail) (433). The age of sheep was estimated by dental examination (Pavaux, 1975). Fecal samples were brought to the laboratory and stored at +4°C until analysis. For the coprological test we placed 5 g of feces in Baermann's apparatus and left them to incubate at 25°C for 24 h. Then, 10–15 ml of the sediment were collected in a tube and centrifuged for 10 min at 1500 rpm. The pellet was examined for the presence of lungworm after adding one drop of Lugol to uncoil the larvae, which were determined under a microscope, based on morphological characteristics.

Parasitological indicators and statistical analysis

The infestation prevalence was calculated as follows: Prevalence = (100 × num. of infested sheep) / (num. of examined sheep)

To study variations in the infestation prevalence according to the age group, breed and month, a chi-square test was performed with Epi Info 6 software (Dean et al., 2011). The threshold value was $p = 0.05$.

RESULTS

Overall infestation indicators

Among the 720 samples of female sheep feces, 68 were infested, corresponding to a prevalence of $9.4 \pm 2.1\%$. There was no significant difference between the infestation prevalence in the two breeds ($p = 0.31$) nor between age groups ($p = 0.64$) (Table I). The infestation prevalence was higher in winter (December to February: $11.7 \pm 4.7\%$) and lower in spring (March to May: $6.7 \pm 3.6\%$). The highest infestation prevalence was reported in December ($25.0 \pm 11.0\%$) ($p = 0.004$; Figure 2).

Infestation prevalence according to lungworm species

The most frequent parasite was *Protostrongylus rufescens* ($4.4 \pm 1.5\%$) followed by *Dictyocaulus filaria* ($2.6 \pm 1.2\%$), *Cystocaulus ocreatus* ($1.3 \pm 0.8\%$) and *Neostongylus linearis* ($1.3 \pm 0.8\%$), and finally *Muellerius capillaris* ($0.3 \pm 0.4\%$). The co-infestation prevalence was very low ($0.4 \pm 0.5\%$) and observed with i) *P. rufescens* and *C. ocreatus*, and ii) *P. rufescens* and *D. filaria*. *P. rufescens* was the most prevalent parasite in both Queue fine de l'Ouest and Barbarine sheep (4.6% and 4.2%, respectively) ($p = 0.78$) (Table I). *P. rufescens* was also the dominant parasite during all seasons except spring (Table I; Figure 3). This species was present during the whole year except in March and April, with a peak in December ($16.7 \pm 9.4\%$) (Figure 4).



Figure 1: Location of Sidi Bouzid District in Tunisia.

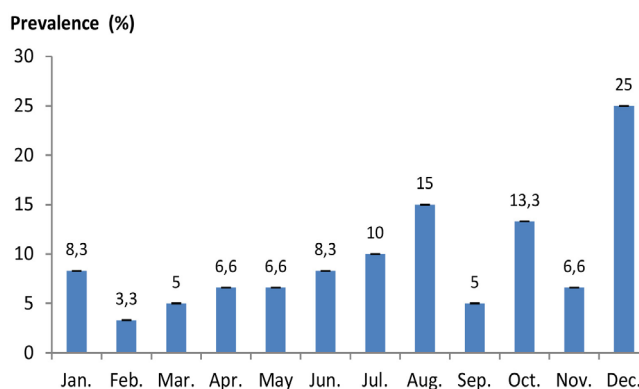


Figure 2: Monthly infestation prevalence by lungworms in slaughtered female sheep in Sidi Bouzid (Central Tunisia).

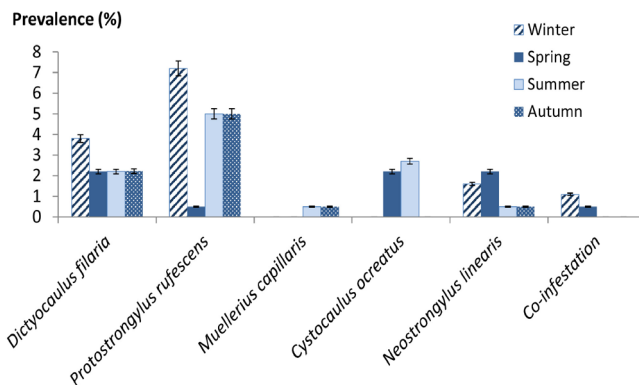


Figure 3: Seasonal infestation prevalence by lungworms in slaughtered female sheep in Sidi Bouzid (Central Tunisia).

Table 1

Infestation prevalence by different lungworm species in female sheep in Sidi Bouzid District (Central Tunisia) according to age, breed and season

Risk factor	Positive/examined (prevalence \pm 95% confidence interval)						Total	P
	<i>Dictyocaulus filaria</i>	<i>Protostrongylus rufescens</i>	<i>Muellerius capillaris</i>	<i>Cystocaulus ocreatus</i>	<i>Neostongylus linearis</i>	Co-infestations		
Age (year)								0.64
≤ 1	8/351 (2.3 \pm 1.6)	15/351 (4.3 \pm 2.1)	0	7/351 (2 \pm 1.5)	5/351 (1.4 \pm 1.2)	1/351 (0.3 \pm 0.6)	34/351 (9.7 \pm 3.1)	
2	5/136 (3.7 \pm 3.2)	6/136 (4.4 \pm 3.5)	1/136 (0.7 \pm 1.4)	0	1/136 (0.7 \pm 1.4)	1/136 (0.7 \pm 1.4)	12/136 (8.8 \pm 4.8)	
3	4/89 (4.5 \pm 4.3)	2/89 (2.2 \pm 3.1)	1/89 (1.1 \pm 2.2)	1/89 (1.1 \pm 2.2)	1/89 (1.1 \pm 2.2)	1/89 (1.1 \pm 2.2)	8/89 (9.0 \pm 5.9)	
4	1/89 (1.1 \pm 2.2)	4/89 (4.5 \pm 4.3)	0	0	1/89 (1.1 \pm 2.2)	0 (6.7 \pm 5.2)	6/89	
> 4	1/55 (1.8 \pm 3.5)	5/55 (9.1 \pm 7.6)	0	1/55 (1.8 \pm 3.5)	1/55 (1.8 \pm 3.5)	0 (14.5 \pm 9.3)	8/55	
Breed								0.31
Barbarine	9/287 (3.1 \pm 2.0)	12/287 (4.2 \pm 2.3)	2/287 (0.7 \pm 1.0)	6/287 (2.1 \pm 1.7)	4/287 (1.4 \pm 1.4)	2/287 (0.7 \pm 1.0)	31/287 (10.8 \pm 3.6)	
Queue fine de l'Ouest	10/433 (2.3 \pm 1.4)	20/433 (4.6 \pm 2.0)	0	3/433 (0.7 \pm 0.8)	5/433 (1.1 \pm 1.0)	1/433 (0.3 \pm 0.5)	37/433 (8.5 \pm 2.6)	
Season								0.32
Winter	7/180 (3.8 \pm 2.8)	13/180 (7.2 \pm 3.8)	0	0	3/180 (1.6 \pm 1.9)	2/180 (1.1 \pm 1.5)	21/180 (11.7 \pm 4.7)	
Spring	4/180 (2.2 \pm 2.2)	1/180 (0.5 \pm 1.1)	0	4/180 (2.2 \pm 2.2)	4/180 (2.2 \pm 2.2)	1/180 (0.5 \pm 1.1)	12/180 (6.7 \pm 3.6)	
Summer	4/180 (2.2 \pm 2.2)	9/180 (5.0 \pm 3.3)	1/180 (0.5 \pm 1.1)	5/180 (2.7 \pm 2.4)	1/180 (0.5 \pm 1.1)	0	20/180 (11.1 \pm 4.6)	
Autumn	4/180 (2.2 \pm 2.2)	9/180 (5.0 \pm 3.3)	1/180 (0.5 \pm 1.1)	0	1/180 (0.5 \pm 1.1)	0	15/180 (8.3 \pm 4.0)	
Total	19/720 (2.6 \pm 1.2)	32/720 (4.4 \pm 1.5)	2/720 (0.3 \pm 0.4)	9/720 (1.3 \pm 0.8)	9/720 (1.3 \pm 0.8)	3/720 (0.4 \pm 0.5)	68/720 (9.4 \pm 2.1)	

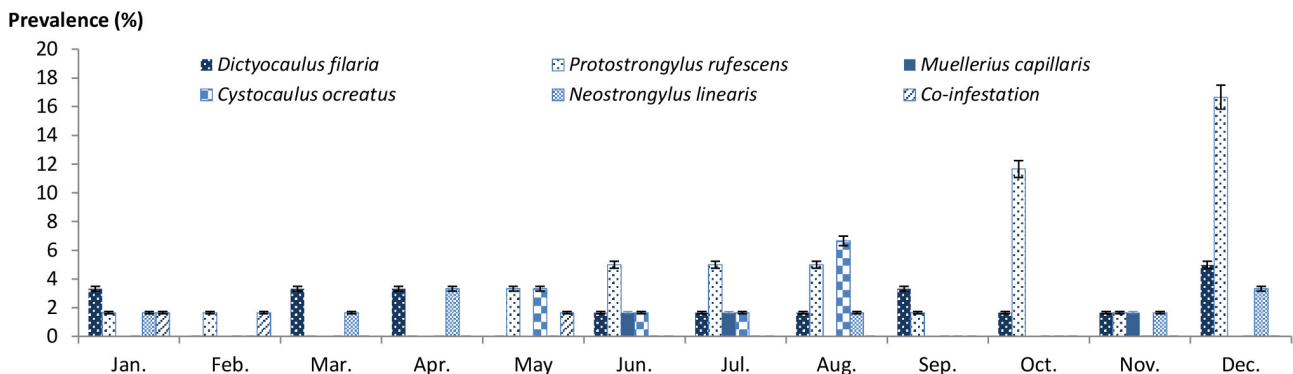


Figure 4: Monthly infestation prevalence by the various lungworm species in slaughtered female sheep in Sidi Bouzid (Central Tunisia).

DISCUSSION

The aim of the study was to assess the prevalence of lungworm infestation in slaughtered female sheep in Sidi Bouzid District (Central Tunisia). In this region, male and female fattened lambs are very popular and

many animals are slaughtered when they are young. Because of drought and illicit import of sheep from neighboring countries in the southern area, sheep meat was in overproduction at the time of the study, and the value of female sheep decreased, prompting farmers to sell them because of high feeding costs. The choice of examining female animals

for this study was thus due to their availability; only few males had been slaughtered in this region at that time, which is rather unusual.

Farmers bringing their animals to the slaughterhouse were questioned to determine the origin of the sheep: only animals reared in Sidi Bouzid District were included in the study. The observed infestation rates therefore reflected the situation in the district. The overall infestation prevalence was estimated at 9.4%.

Until the present study, in Tunisia lungworm infestation prevalence in sheep had only been assessed by examining lung lesions during studies carried out by veterinary students. The prevalence reported in the present study was lower than those reported in Tajerouine, Northwest Tunisia (12.5%; Hammami, 1982) and Médenine, Southeast Tunisia (15.1%; Temri, 2007), similar to that recorded in Kasserine, Central West Tunisia (9.3%; Aloui, 2005), and higher than that reported in Sfax, Southeast Tunisia (7.3%; Ben Said, 2008). These variations could be explained by several factors: i) genetic resistance of animals, as it was shown that there is a difference in sheep genetic resistance to parasites (Bedhiaf-Romdhani et al., 2008; Sassi-Zaidy et al., 2014); ii) differences in breeding management from one region to another and in several cases from one farm to another (Atti et al., 2004; Ben Salem et al., 2011); iii) differences in environmental conditions leading to a high disparity in the resistance of the free larval stages and in the activity dynamics of intermediate hosts (Yildiz, 2006); and iv) the use of ivermectin that can reduce infestation. The low infestation prevalence of ewe lambs could be explained by the intensive breeding system. Indeed, Sidi Bouzid District is a steppe with poor grazing areas; the animals are therefore mainly bred under a zero grazing system.

Since sheep are continuously kept in barns with almost no grazing periods, it is rather surprising to notice that they are infested by protostrongylidae parasites as well as by *D. filaria*, because the latter has a direct life cycle although the transmission of *P. rufescens*, *C. ocreatus*, *N. linearis* and *M. capillaris* involves snails as intermediate hosts. In barns, free stages of lungworms (i.e. infecting larvae of *D. filaria*) are protected against desiccation and sun light action. On the other hand, as the infestation by protostrongylidae is related to the availability of their intermediate hosts, it was unexpected to find these species, at least during the dry season. This could be explained by the fact that sheep may harbor residual parasite populations, which infested animals during the rainy season. However, this point needs to be investigated.

The lungworm prevalence in Sidi Bouzid District was similar to those reported in Iran (10.8%; Borji et al., 2012) and in Spain (11.6%; López et al., 2011), but lower than those reported in Turkey (14%; Yildiz, 2006), in Morocco where the animals were infested by *M. capillaris* (35.5–70%) and *D. filaria* (5–30%) (Paliargues et al., 2007), and in Ethiopia (Addis et al., 2011) where a prevalence of 32.6% was observed. In this latter country, animals are reared in highlands where the climate is quite different: mean annual rainfall of 950–1750 mm and temperatures of 3–21.6°C. In Morocco, the high infestation prevalence by *M. capillaris* was recorded during autumn (69%) and winter (42%) and could be explained by the low temperature leading to a higher survival rates of free instars (larvae). The high density of sheep sharing common pastures, because extensive breeding systems are continuously used throughout the year, could also explain the high prevalence observed.

Borji et al. (2012) reported the presence in Iran of two lungworm species, *D. filaria* and *P. rufescens*. They showed that the infestation prevalence was significantly higher in young animals than in adult sheep (5.9% and 0.8%, respectively). Conversely, the present study revealed no significant difference between age groups. Borji et al. (2012) suggest that sheep could acquire immunity against respiratory nematodes with age. In the present study, the absence of difference between the infestation prevalence in young and adult sheep

was probably due to the fact that young animals did not graze and were not regularly infested by worms, preventing the acquisition of immunity.

This survey provided information about seasonal infestation dynamics by several lungworm species; sheep were more infested in winter. There is however a gap in the knowledge of the economic impact of infestation by these parasites. A cost-benefit analysis should be carried out to decide whether a control program should be implemented in Tunisia. Both cost-benefit analysis and preventive anthelmintic treatments should be based on the epidemiological data reported in this study.

Acknowledgments

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Resumé

Elati K., Aloui S., Dhibi M., Rekek M., Gharbi M. Variation saisonnière de l'infestation de brebis par les strongles respiratoires à l'abattoir de Sidi Bouzid (Tunisie centrale)

Les strongyloses respiratoires sont parmi les plus importantes maladies affectant les ovins élevés dans divers systèmes d'élevage de nombreux pays. En Tunisie, la lutte contre ces parasites est conduite par les éleveurs sans aucun fondement scientifique. Une enquête a été réalisée dans l'abattoir du gouvernorat de Sidi Bouzid (centre du pays) afin d'évaluer la prévalence d'infestation des moutons par les strongles respiratoires. L'étude a duré 12 mois et a concerné 720 des brebis abattues. La matière fécale de chaque brebis a été recueillie et examinée par la technique de Baermann. Soixante-huit échantillons étaient infestés par des larves de nématodes pulmonaires ($9,4 \pm 2,1$ %). La population était dominée par *Protostrongylus rufescens* ($4,4 \pm 1,5$ %) suivie de *Dictyocaulus filaria* ($2,6 \pm 1,2$ %), *Cystocaulus ocreatus* et *Neostrongylus linearis* ($1,3 \pm 0,8$ %), et enfin *Muellerius capillaris* ($0,3 \pm 0,4$ %), alors que les infestations mixtes concernaient $0,4 \pm 0,5$ % des échantillons. L'infestation par les strongles a été observée dans tous les groupes d'âge sans différence significative ($p = 0,64$). Il n'y a pas eu, non plus, de différence d'infestation significative entre les brebis de races Barbarine ($10,8 \pm 3,6$ %) et Queue fine de l'Ouest ($8,5 \pm 2,6$ % ; $p = 0,31$). La prévalence la plus faible a été enregistrée au printemps ($6,7 \pm 3,6$ %) et la plus élevée en hiver ($11,7 \pm 4,8$ %), mais la différence entre les saisons n'était pas significative ($p = 0,32$). Cette étude concernant la dynamique d'activité des strongles pulmonaires devrait être complétée par des suivis permettant de déterminer l'importance économique de ces infestations, afin d'offrir une base solide à la mise en place de programmes de lutte spécifiques contre ces parasites en Tunisie.

Mots-clés : ovin, brebis, Protostrongylidae, Nematoda, variation saisonnière, Tunisie

Resumen

Elati K., Aloui S., Dhibi M., Rekek M., Gharbi M. Variaciones estacionales de la infestación de parásitos pulmonares en ovejas en el matadero de Sidi Bouzid (Túnez central)

La estrogilosis respiratoria está entre las enfermedades más importantes que afectan a las ovejas criadas en varios sistemas de producción en varios países. En Túnez, el control de estos parásitos lo llevas a cabo los finqueros sin formación científica. Una encuesta llevada a cabo en el matadero del distrito de Sidi Bouzid (Túnez central), para evaluar la prevalencia de la infestación de estos parásitos pulmonares en ovejas. El estudio duró 12 meses e involucró 720 de las ovejas sacrificadas. Se recolectaron muestras fecales de cada oveja y se examinaron mediante la técnica de Baermann. Sesenta y ocho muestras estuvieron infectadas con larvas de nemátodos pulmonares ($9,4 \pm 2,1\%$). La población estuvo dominada por *Protostrongylus rufescens* ($4,4 \pm 1,5\%$), seguida de *Dictyocaulus filaria* ($2,6 \pm 1,2\%$), *Cystocaulus ocreatus* y *Neostrongylus linearis* ($1,3 \pm 0,8\%$), y finalmente *Muellerius capillaris* ($0,3 \pm 0,4\%$), mientras que las infestaciones mixtas concernieron $0,4\% \pm 0,5\%$ de las muestras. La infestación de parásitos pulmonares se observó en todos los grupos etarios sin diferencia significativa ($p = 0,64$). Además, no hubo diferencia significativa en la infestación entre razas ovinas Beréberes ($10,8 \pm 3,6\%$) y Cola Fina del Oeste ($8,5 \pm 2,6\%$; $p = 0,31$). La menor prevalencia se registró en la primavera ($6,7 \pm 3,6\%$) y la más alta en invierno ($11,7 \pm 4,8\%$), pero la diferencia entre las estaciones no fue significativa ($p = 0,32$). Este estudio concerniente a la dinámica de la actividad de los parásitos pulmonares debe ser complementado con un proyecto de monitoreo para determinar la importancia económica de estas infestaciones y proveer bases sólidas para el establecimiento de programas de control específicos contra estos parásitos en Túnez.

Palabras clave: ovino, oveja, Protostrongylidae, Nematoda, variación estacional, Túnez

