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STUDY ON STRONGYLE INFECTION AND OTHER ASSOCIATED PARASITES IN SHEEP, IN SOUTHERN AREA OF ROMANIA

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Abstract

Parasitism of diferent strongylid nematodes belonging to the families Trichostrongylidae, Strongylidae and Ancylostomidae on sheep, are widespread in all regions of animal breeding, but especially in areas with a humid climate. As they result in drastic decreases in production of adult animals and growing delays in young animals, imposing costly prophylaxis measures, these parasitic diseases have a significant economic impact. While studies in helminthology are directed towards more in-depth perspectives, such as DNA modifications of parasites, the continuous monitoring of occurrence and prevalence of these parasitic species remains important. In addition, because there are few reports on gastrointestinal parasites of ruminants and especially of sheep in our country, the present study aims at investigating the occurrence of strongyle infections in sheep, in Southern Romania. For this, a coproparasitological study was carried out on a total number of 424 sheep, out of a population of 3256 animals, originating from six farms from this region. A flotation technique was used to detect helminth eggs; however, protozoa oocysts, when detected, were also registered; additionally a Baermann method, for detection of lungworm larvae was performed. The findings indicate the following infections, as follows: strongyles 65.1%; Moniezia spp. 18.9%; Eimeria spp. 14.4%; Protostrongylidae 7.1%, Dictyocaulus filaria 2.1%. The findings highlight the high occurrence of strongyle infections, but also of other parasitic species with significant impact on the both animal health and their productivity and highlight the importance of a proper parasitological control to be applied in sheep farms.

Key words*: sheep strongyles, prevalence, Southern Romania.*

INTRODUCTION

Parasitism of digestive strongyle on sheep is widespread and causes important economic losses, with a significant impact on the sheep industry worldwide, including Romania. Recent studies in the East (Kumar et al., 2015; Mitrea, 2011; Rajpoot et al., 2017), Middle-East (Gholami et al., 2015; Sharifdini et al., 2017) and France (Arece-García et al., 2007; Mokhtar et al., 2009) have shown a greater interest towards this subject, thus enriching the knowledge in the field. While, surely, parasitic infections worldwide will be based, more or less, on the same issues, it is the prevalence of some and not other, and the particularity of certain variables that determines the degree and specifics of parasitism in a country or area. Furthermore, while current studies of helminthology have become increasingly focused on various DNA manipulations of parasites (Horak, 2019; Marchiondo et al., 2019; Wang et al., 2013), there are still of high interest epidemiological studies, which are the basis for developing sustainable parasitological control programs.

It is widely acknowledged that gastro-intestinal strongyle infections cause important economic costs to sheep breeders in terms of production, reproduction, weight gain and, not least, mortality (Odoi et al., 2007).

Evidently, the impact on animal welfare is exponential. In this context, early diagnosis and farm management, understood as using proper prophylactic measures, are vital to maintain the lowest levels of helminths' incidence.

While, a significant number of studies have recently reported on the occurence and prevalence of endo-parasites in Romanian horses (Madeira de Carvalho et al., 2008; Covasa and Miron, 2011; Ionita et al., 2013; Cernea et al., 2015; Buzatu et al., 2014; 2016; Morariu et al., 2016), information about the epidemiology of gastro-intestinal parasites in sheep is still parsimonious.

Due to the fact that our country ranks the 4th place in the EU regarding sheep flocks, it is necessary for continuous updating studies in

the field. The present study aimed to determine the presence of gastro-intestinal strongyles and other associated endo-parasites on sheep in South of Romania, as well as the factors influencing the presence of these, in order to characterize the pattern of parasitism in the area.

MATERIALS AND METHODS

The study was carried out on a representative batch of 424 sheep, from a population of 3256 sheep, between the years 2012-2016. The investigated farms consisted of three agrozootechnical units (AIC, FS, OM) and three private households (SC, AM, BA), in Southern Romania.

The batch comprised approximately 85.14% females (lactating, pregnant), aging over 2.5 years old and the rest, lambs, male and female youth, and adult males; the age of animals varied between 2.5 months and 5 years . The sheep included in the study were of the following breeds: Tigae, German Blackhead, Merinos Palas, Teleorman Carabas, and Texel.

Fecal samples were collected from individual animals, in 3 series, in all seasons.

The following methods and analyses were performed:

- coproparasitological investigations for detecting eggs of digestive parasites werecarried out, by flotation (Willis technique). Aditional, a larvoscopic technique (Baermann method) was performed (Ionita and Mitrea, 2013). These investigations were performed in the Laboratory of Parasitology of the Faculty of Veterinary Medicine, USAMV Bucharest. The information was coded into SPSS (Statistical Package for Social Sciences), with a confidence interval of 95%. While each of the homesteads had differentf lock numbers, equivalent examples were taken (cca 70 for each ranch) to maintain a strategic distance from critical varieties in results.
- anatomopathological analyses, with samples taken promptly after evisceration and necropsies from the small and thick intestine, and rennet.

The parasites' prevalence was evaluated using the Frequencies and Crosstabs commands in SPSS. The factors potentially influencial of gastrointestinal infections were explored through Correlations commands and One-Way ANOVA analyses.

Discussions were held with the owners and farm administrators about the frequency of prophylactic treatments anytime within the period of research.

The variables introduced into the statistical analysis to evaluate the parasites' incidence consisted of: the sheep age range (1-18 months; 19-60 months) and race; the season; and year in which the samples were collected; the use of prophylactic treatments; the presence of associated parasitism; the farm type (agrozootechnical, individual household).

RESULTS AND DISCUSSIONS

Overall, a proportion of 69.8% (296/424) of the animals included in the study were positive for at least one parasite infection (Table1).

Table 1. Parasite infection levels per each farm included in the study

Animals	Prevalence of parasite infection in sheep by farm					
	AIC	FS	OM	SC	AM	BA
Nr. Sampled	70	70	70	74	70	70
Nr. (%) positive	49 (70%)	18 (25.71%)	35 (50%)	64 (86.48%)	60 (85.71%)	70 (100%)

The coproparasitological examinations revealed the presence of protozoan oocysts, eggs of gastrointestinal helminth parasites and larvae of lungworms (Figures 1-5), as following: GI-strongyles, *Eimeria* spp., *Moniezia* spp., *Protostrongylus* spp., *Dictyocaulus filaria*.

Figure 1. Digestive strongyle (eggs) in sheep (ob. 10x)

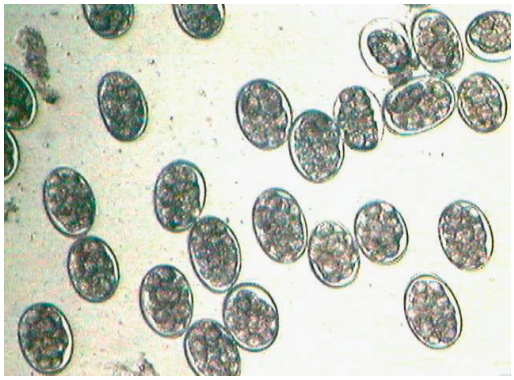

Figure 2. Heavy infection with digestive strongyle (eggs) in sheep (ob. 20x)

Figure 3. Mixt infection: digestive strongyle (eggs) and *Eimeria* (oocysts) in sheep (ob. 20x)

Figure 4. Larva of *Dictyocaulus filaria* (lungworm) in sheep (ob. 10x)

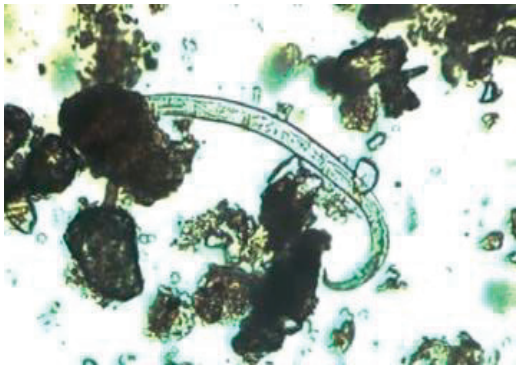

Figure 5. Protostrongylid larva (lungworm) in sheep (ob. 10x)

The prevalence of the parasite species identified in sheep is presented in Table 2.

Table 2. Prevalence of parasite species in the sheep included in study (frequencies and percentages)

Parameter/ locality	**Parasite species identifiied**				
	GI-strongyles	*Moniezia* spp.	*Eimeria* spp.	*Protostrongylids*	*Dictyocaulus filaria*
Total Number of infected animals	276	80	61	30	9
Percentages	65.1%	18. 9%	14.4%	7.1%	2.1%
	Number of positives animals per farm (frequencies and percentages)				
AIC (n=70)	47 (67.1%)	2 (2.85%)	17 (24.3%)	0 (0%)	4 (5.79%)
FS (n=70)	16 (22.9%)	13 (18.6%)	5 (7.1%)	2 (2.9%)	0 (0%)
OM (n=70)	32 (45.7%)	3 (4.3%)	10 (14.3%)	0 (0%)	3 (4.3%)
SC (n=74)	64 (86.5%)	42 (56.8%)	0 (0%)	7 (9.5%)	0 (0%)
AM (n=70)	52 (74.3%)	13 (18.6%)	18 (25.7%)	8 (11.4%)	0 (0%)
BA (n=70)	65 (92.8%)	7 (10.0%)	11 (15.7%)	13 (18.6%)	2 (2.85%)

Concerning the incidence by age, adults presented infections in proportion of 65.02% compared to the youth, in 98.36% of the specific herds. The proportions are statistically significant (p = -255) and illustrate that parasitism is inversely proportional to age: the younger the specimens, the greater the likelihood of parasites existence.

This result is consistent with the existing literature, including recent studies in Romania that emphasize the predisposition of youth to parasitism, but to varying degrees: in a study conducted in Cluj County, Negrea et al. (2013) reported the presence of strongyles in the range of 72.7% in youth and 65% in adults. In our study, the youth presented infections with strongyles in proportion of 95.1%, and adults, 60.1%, data which are correlated significantly (p = -258).

The periods of spring (26%, N = 424; df = 3) and autumn (20%, N = 424; df = 3) had the most frequent cases of parasitism, especially infections with strongyles: 22.6% spring, 19.3% autumn. In summer and winter, parasitic infections were generally identified in proportion of 14%, respectively 10%, and with strongyles particularly, in proportion of 13.2%, respectively 9.9%. The correlations in this respect were statistically significant (p = -253).

Of the cases of parasitism prevalent, the male youth was affected 100%, the female youth, in

96.77%, and the adult females, in 64.85% (df = 1). The results were significantly influenced by the type of farm: the individual households had very high levels of infection (65.5%), one of them reaching 100% degree of infection, while the agrozootechnical farms showed lower levels (34.5%, SD = 0. 47).

Prophylactic treatments were administered to 24.3% of the specimens (n = 424; SD = 0.42), however there were large variations between farms, as in the case of parasites' incidence: agrozootechnical farms performed prophylactic treatments in proportion to 17.45%, while the individual households, 6.83% (SD = 0.45). The FS farm differentiated itself as a role-model, with the lowest level of parasitism, as well as with the highest frequency of prophylactic treatments. In addition, the use prophylactic treatments represented a significant variable influencing parasitim (p =.157).

The incidence of parasites in the sample was, as follows: strongyles - 65.1%, *Moniezia* spp. - 18.9%, *Eimeria* - 14.4%, *Protostrongylus* spp. - 7.1%, *Dictyocaulus filaria* - 2.1% (N = 424).

Among the detected parasitoses, numerous infections were mixed: while there was a significant percentage of simple infections with strongyles (31%) and *Moniezia* (15%), there were important proportions of mixed infections of strongyles with *Moniezia* spp. (15%), strongyles and *Eimeria* (12%), strongyles and *Protostrongylus* (4%); the rest being infections with *Eimeria* and *Protostrongylus* spp. (2%); and strongyles with *Dictyocaulus filaria* (2%, N = 424).

In a study conducted on sheep flocks from Northern and Southern Romania, Mitrea et al. (2008) indicated the presence of strongyles in relation to other parasitic populations, in a range of 71.5% to 92.8%; and *Moniezia*, between 14.2% and 21.4%. The results of the present study are close to these values, through the presence of strongyles in proportion of 65.1%; and converge in what concerns *Moniezia* spp., with an incidence of 18.9%.

At the same time, the results of our research converge with those highlighted by Mitrea et al. (2008) additionally in what regards the prevalence of parasitic associations with two species: the cited study indicates an interval between 14.28% to 35.7%, and our empirical approach describes a percentage of 35% of the sample. However, our research did not reveal associations with more than two parasitic species, nor any species other than those discussed, and the prevalence of *Eimeria* is different (up to 50%) (Mitrea et al., 2008), compared to 14.4%, in the present study.

The gender was a significant variable at the level of the whole sample. As in the recent literature in our country, this variable has not been signalled as statistically significant in sheep, but rather in other geographical areas - such as, for example, sheep genderin the Punjab area (Singh et al., 2017), future studies will have to further assess the validity of these significant values on larger samples from Romania.

Similarly, performing prophylactic treatments was another significant variable in the influence on parasitism, which has not been reported as significant in sheep in Romania, but was indicated as such in countries such as Brazil (Machado et al., 2019).

While age and prophylactic treatments are significantly correlated with parasitism level, the type of farms remains variable with the strongest impact (Mitrea, 2002), a fact that emerges from the highest level of correlation. Thus, as Indre et al. (2011) outlines, parasitic situations differ greatly from one farm to another. FS stood out as a standard model in terms of the low level of parasitism (25.7% of its own sample) (Table 1), as well as in the use of prophylactic treatments (51.42%). All variables indicated here as significant are predictors of parasitism in sheep in analyzed sample in the range 34% (R^2 = .377).

CONCLUSIONS

To conclude, in the interval between 2012-2016, what seems to have been specific for the Southern area of Romania is the prominent presence of strongyles, in both single and mixed infections and the most important variable influencing this was the type of farm sheltering the animals.

Beyond the variables discussed in the analysis, there is, undoubtedly, a management component of the grazing and sheltering of the sheep that may determine the presence or absence of parasitism; and which can justify

the different levels of parasitism in the 6 farms. The fact that there is a significant and strong correlation between the types of farms sheltering the sheep and parasitism can be attributed to additional prophylactic measures, unaccounted for by our approach.

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