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Gastrointestinal parasites as a possible threat to an endangered autochthonous Portuguese sheep breed

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Research Paper

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Abstract

Helminth and protozoan infections are responsible for important diseases in grazing sheep, which can be especially threatening in an autochthonous breed at risk of extinction like the Churra Galega Mirandesa Portuguese sheep breed. The aim of the present study was to determine the diversity, prevalence and burden of gastrointestinal parasites in these sheep and to assess the effects of deworming practices, cohabiting animals on the farm and feed management. Coprological qualitative and quantitative analysis (flotation, natural sedimentation and McMaster method) were used to identify and quantify gastrointestinal parasites and a questionnaire was designed and applied. A total of 512 faecal samples were collected from 49 flocks, and 49 replies to the questionnaire were received. Parasites were identified in 100% of the flocks, and in 97% of the samples. The genera or species that have been morphologically identified were: strongyle-type, *Nematodirus* spp., *Skrjabinema* spp., *Moniezia expansa*, *Moniezia benedeni*, *Trichuris* spp., *Capillaria* spp., *Eimeria* spp., *Dicrocoelium* spp. and *Fasciola hepatica*. This is the first report in Portugal of *Skrjabinema* spp. The burden of parasites' oocysts and eggs per gram in faecal samples ranged, respectively, from 50 to 17,550 for *Eimeria* spp., and from 50 to 6250 for strongyle-type eggs. Factors affecting parasitic infections were evaluated using a multivariate logistic regression. Grazing time and a lack of anthelmintic treatment were positively associated with *Nematodirus* spp. infection. This study showed that there is a high prevalence and diversity of gastrointestinal parasites in the Churra Galega Mirandesa sheep breed.

Introduction

The conservation, sustainable use and promotion of animal genetic resources (AnGR) have been a priority in Portuguese and European Union (EU) policies. Over a number of years, the EU and the Portuguese Ministry of Agriculture have worked to keep AnGR and developed actions in collaboration with breeders associations for the protection, conservation and improvement of animal genetic heritage. Autochthonous breeds are a very valuable genetic resource owing to their biodiversity and can be distinguished for their ability to adapt to very adverse environmental conditions, to poor feed availability and also for their tolerance to parasitic infections (Paim *et al*., 2013; McManus *et al*., 2014). Livestock farming is central to the sustainability of rural communities worldwide (Morgan *et al*., 2013), and a large part of the sheep production in Portugal, like in many other countries, is based on extensive systems (Montossi *et al*., 2013), taking advantage of forage resources in less favourable areas that would otherwise not be used. Sheep population in Portugal was estimated to be approximately two million in 2016 (FAOSTAT, 2016). Portugal has a range of particularities, with an enormous variability of orography, soil, climate, land structure, social and cultural traditions, that allow for a considerable diversity of genetic resources, such as a significant number of autochthonous breeds, namely of sheep. The Churra Galega Mirandesa (CGM) sheep is a Portuguese autochthonous breed raised in the north-east of Portugal, well adapted to the geographic and climatic conditions, with a great aptitude for meat production, with wool being used mainly for handicraft and milk being consumed only by lambs.

CGM sheep graze most of the year, because of the climatic conditions with continental and Atlantic influences. Such sheep are raised in extensive systems, fed on cereal stubble and natural pastures (characterized mainly by annual grass species), and most of them are only

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brought into the paddocks during the night. Consequently, these sheep are extremely exposed to a wide range of gastrointestinal (GI) helminths and protozoa (Fox *et al.*, 2013; Kumar *et al.*, 2013; Rinaldi *et al.*, 2015a).

In European countries, a range of Strongylida (*Teladorsagia circumcincta*, *Haemonchus contortus*, *Trichostrongylus* spp., *Nematodirus battus*, *Cooperia* spp., *Chabertia* spp. and *Oesophagostomum* spp.) have already been identified in usually mixed-parasitic infections in sheep (Fox *et al.*, 2012; Zajac & Conboy, 2012; Bowman, 2014; Rinaldi *et al.*, 2015b), but ruminants also carry large numbers of protozoa species (Taylor, 2000; Chartier & Paraud, 2012).

GI parasitism and the subsequent host immune response have important consequences for sheep production, associated with reduced nutrient utilization, growth rate and milk production (Arsenos *et al.*, 2007; Jacobson *et al.*, 2009; Rinaldi *et al.*, 2015b). The subclinical parasitic infections are responsible for significant economic losses in animal productivity (Morgan *et al.*, 2013; Roeber *et al.*, 2013). This research focuses on the lack of well-established data on the prevalence and distribution of sheep GI parasites in the north-east of Portugal in general, and in the CGM sheep breed in particular. Therefore, this study was designed to assess the diversity, the prevalence and burden of GI parasites in this autochthonous Portuguese sheep breed. Moreover, we evaluated the predictability of some risk factors, such as grazing type, cohabitant animals and the parasite-control practices on the GI parasitic prevalence and burden.

Material and methods

Study area

The study was conducted between September and December 2016 and included sheep flocks located in the north-east of Portugal. For this purpose, we joined the association of the breeders of CGM sheep (ACOM), the officially recognized entity for the CGM sheep breed herd-book management, and visited their CGM sheep flocks. The definition of the sample was, therefore, of convenience sampling. According to the ACOM, the CGM includes 6763 females and 170 males approximately, but the number has been decreasing and the breed has been classified as an endangered autochthonous breed (DGAV, 2013). The traditional sheep production system is extensive and most flocks are maintained outside, within fenced areas for sheep grazing, and are only brought into the paddocks during the winter and cold days. Sheep graze on natural pastures, and it is common for flocks to graze in community pastures. The flocks were sampled from four municipalities of the Bragança district (Miranda do Douro, Vimioso, Mogadouro and Bragança) (fig. 1), which have similar climatic conditions with continental and Atlantic influences, characterized by cold winters and warm summers.

Faecal sample collection and analysis

The sample size expected for this study was determined by using a confidence level (CL) of 95%, an error (D) of 5% and an expected prevalence (P) of 20%, by calculating $Z^2(P(1-P))/D^2$, where the value of the standard normal distribution (Z) is Z = 1.96 for CL 95%. So, the required number of sampled sheep was of 246 or more.

The geographical coordinates and elevation data of farm locations were registered. Fresh faecal samples were collected from sheep older than nine months of age, directly from the rectal ampoule/rectum or immediately after defecation. The samples were stored in individual plastic bags, and labelled with animal identification number, age, sex, day/month of collection and farm location, and transported and maintained under 4°C until the arrival at the laboratory. Each sample was first examined macroscopically for the possible detection of proglottids.

All individual samples were analysed using three methods: flotation in saturated sodium chloride solution (Willis), natural sedimentation technique for qualitative assessment and the McMaster method (quantitative) (Zajac & Conboy, 2012). In terms of the burden of infection, they were classified as low, moderate and high when the number of eggs/oocysts per gram of faeces (EPG/OPG) was up to 1000, between 1001 and 2000, and above 2001, respectively (Soulsby, 1988).

Data collection

The design of the questionnaire followed a literature review about sheep breeding and management, which was subsequently tested and reviewed with regard to the terms used. This resulted in a final questionnaire composed of 13 questions, related to flock size, cohabitant animals, food management and deworming practices and procedures, number of treatments/year and date of last treatment. Body condition (Bc) was recorded according to Ducanson (2012), based on five scores.

The questionnaire was administered to every farm at the same time as faecal samples were collected.

Statistical analysis

The prevalence (P) was calculated by P = n/N, where n = number of samples positive to different species of GI parasites and N = total number of samples analysed. Analyses were conducted using SAS® (SAS Institute, 2004). The association between the prevalence of the various parasites and independent variables, like municipality, month of collection, grazing type, deworming, cohabitation with other animals (cattle, goats, donkeys, horses and dogs), Bc and the total number of animals on the farm (the latter two were considered as co-variables) were evaluated using the Chi-square test (χ^2). The CL was held at 95% and 99%; $P < 0.05$ and $P < 0.01$ was set for significance.

Initially, frequencies were analysed through the PROC FREQ of SAS (SAS Institute, 2004). Subsequently, the probability of presence/absence of the various parasites expressing variability (strongyle, *Nematodirus* spp., *Eimeria* spp., *Moniezia benedeni*, *Dicrocoelium* spp. and *Trichuris* spp.) was analysed by logistic regression through PROC LOGISTIC of SAS (SAS Institute, 2004). Strongyle, *Nematodirus* spp. and *Eimeria* spp. were subjected to analysis of variance through PROC GLM of SAS (SAS Institute, 2004) and the least square mean (LSM) was estimated. The χ^2 and analysis of variance one-way tests were used respectively to compare the prevalence and the mean intensity of the different GI parasites infections.

Results

Questionnaires

Cooperation with the sheep farmers was satisfactory and all of them participated in the questionnaire. A total of 49 replies to the questionnaires were received, one for each flock sampled. Most farms (48.9%) have less than 130 sheep and only two (4.1%) do not have other cohabiting animals; dogs are almost

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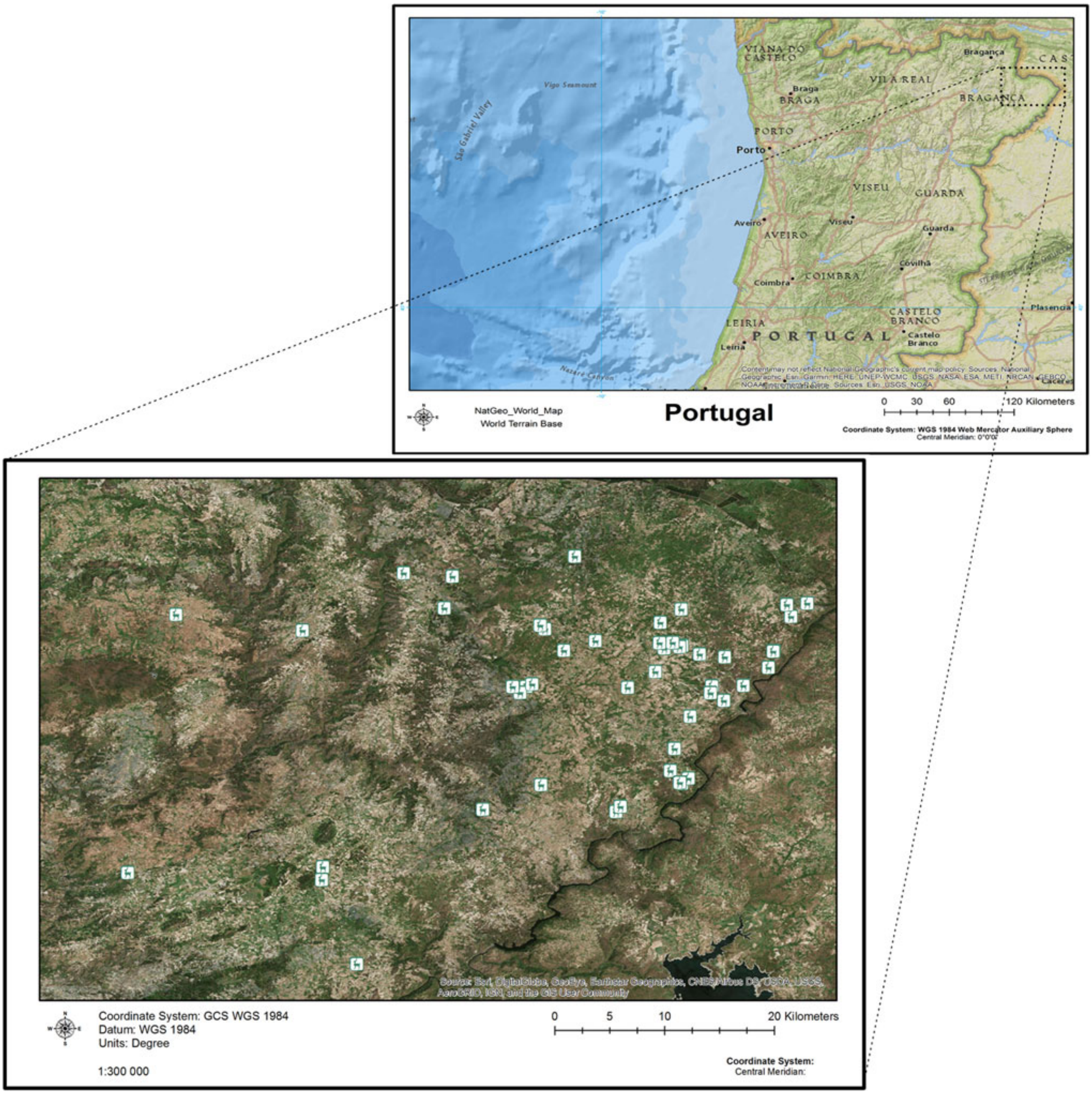

Fig. 1. Localization of the flocks sampled in the study area.

ubiquitous (93.9%) and donkeys are very common (24.5%). The vast majority of flocks (81.6%) graze on community pastures. Most farmers (75.5%) report deworming (table 1).

Parasite prevalence

A total of 512 faecal samples from 49 flocks were analysed and in 97.1% (497/512) one or more GI parasites have been identified. The overall prevalence of the parasites found and the results per parasite for each municipality are presented in table 2. Nematoda, Cestoda, Trematoda and Protozoan parasites have been identified. Strongyle-type eggs (85.4%) were the most prevalent parasites. Miranda do Douro and Vimioso were those where a greater diversity of parasites was found, namely *Dicrocoelium* spp. *Fasciola hepatica* was only identified in Miranda do Douro.

The infection burden based on faecal eggs count (FEC) ranged from 50 to 6250 EPG for helminths and from 50 to 17550 OPG for protozoa. The mean, range of EPG and level of infection per sample are presented in table 3.

In relation to the burden of the GI parasites, the study revealed that the mean EPG was notably low for all species of GI parasites observed.

Analysis of association of parasite prevalence and environmental factors

The association between each GI parasite and the factors studied is presented in table 4. χ^2 shows the degree of association with

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Table 1. Characterization of flock size, other animal cohabitation, grazing time, feeding and deworming frequency.

Variable	Categories	*n*	%
Flock size	<65	11	22.4
	66–130	13	26.5
	131–200	10	20.4
	201–300	6	12.2
	301–450	4	8.2
	>450	5	10.2
Cohabitant animal	Cattle	9	18.4
	Goats	6	12.2
	Donkeys	12	24.5
	Horses	3	6.1
	Dogs	46	93.9
	None	2	4.1
Grazing time	All day	24	49.0
	Early morning + late afternoon	25	51.0
Grazing in community pastures	Yes	40	81.6
	No	9	18.4
Sheep feeding supplements[a]	Yes	30	61.2
	No	19	38.8
Anthelmintic treatment	Yes	37	75.5
	No	12	24.5
Last year deworming frequency	One treatment		
	one year ago	6	12.2
	half a year ago	23	46.9
	in the last three months	6	12.2
	Two treatments (in this year)	2	4.1

[a]Sheep feeding supplements are defined as food with a lower amount of fibre and more energy and/or protein for sheep.

independent factors, such as municipality, month of collection, type of pasture, deworming, cohabitation with animals (cattle, goats, donkeys, horses and dogs), Bc and flock size.

In most of the GI species found, there was no difference in prevalence according to the variables shown in table 4, but when analysing the different variables that interfere with GI parasites infections, it was observed that the month of sampling, grazing time, deworming, the presence of cohabitant animals (like dogs) and Bc were the variables that exerted significant influences on the occurrence of some GI parasites.

Statistical differences in the results obtained were found between the periods of grazing. Table 4 shows that there were no significant differences in distribution between the municipalities in the study area and flock size ($P > 0.05$).

When sheep graze during all day, the risk of the infection by *Nematodirus* spp. is 1.5 higher (odds ratio (OR) = 1.515, $P < 0.05$). Non-dewormed sheep are 1.6 times (OR = 1.594, $P < 0.05$) more likely to have *Nematodirus* spp. infection. Grazing time is also associated with occurrence of *Trichuris* spp.: when grazing occurs during some hours in the early morning and late afternoon, the risk of infection with *Trichuris* spp. is 2.2 times higher (OR = 2.183, $P < 0.05$).

In the case of *Dicrocoelium* spp., when grazing during all day, animals are 2.2 times more likely to be infected by *Dicrocoelium* spp. (OR = 2.164, $P < 0.01$). The month of sampling was significantly associated with the occurrence of *M. benedeni* ($P < 0.05$) and *Dicrocoelium* spp. ($P < 0.01$). The risk of occurrence of *M. benedeni* and *Dicrocoelium* spp. is 5.9 and 3.9 times higher, respectively, in October.

Bc seemed to have a significant association with the prevalence of *Eimeria* spp. ($P < 0.01$) (table 5): *Eimeria* spp. was higher in sheep with poor Bc and increasing Bc was significantly associated with decreased odds of *Eimeria* spp. infection.

The strongyle and *Nematodirus* spp. FEC were significantly influenced by deworming ($P < 0.05$) and Bc ($P < 0.01$).

The LSM of FEC in strongyle and *Nematodirus* spp. shows significant differences between deworming and non-deworming.

The LSM of FEC in dewormed sheep is lower (231.78 ± 27.41) than in non-dewormed sheep (361.09 ± 51.24). No significant variation was seen between deworming and *Eimeria* spp. The Bc of sheep is influenced by the burden of strongyle, *Nematodirus* spp. and *Eimeria* spp.

For each plus one in Bc, the amount of strongyle, *Nematodirus* spp. and *Eimeria* spp. decreases −152.20 ± 41.45, −10.39 ± 2.71 and −295.34 ± 92.31, respectively.

Discussion

The current study is the first GI parasites survey in CGM sheep breed in their original location and aim to document the prevalence, diversity and burden of helminth and protozoa in those flocks.

The results of the survey clearly evidence a high prevalence of GI helminths. The diversity found in this survey could be higher if we had carried out coprocultures to distinguish different strongyle genera or species eggs. These parasites, alone or in association, can be responsible for negative effects on sheep health and production (Bowman, 2014).

The observed strongyle-type eggs prevalence rates did vary between the municipalities. Different studies performed in the north-west of Spain, in a region situated close to the one considered in our study, with similar geographic elevation and climatic conditions, showed a higher prevalence of strongyle and *F. hepatica* has been sporadically found (Pedreira *et al.*, 2006; Martínez-Valladares *et al.*, 2013; Atlija *et al.*, 2016).

Strongyle and *Eimeria* spp. were the most common parasites found, similarly to what has been observed in previous studies, not only in Europe (Torina *et al.*, 2004; Skirnisson, 2007; Idris *et al.*, 2012; Kantzoura, 2012; Rinaldi *et al.*, 2015a) but also in other continents (Kumar *et al.*, 2015; Tramboo *et al.*, 2015; Kelemework *et al.*, 2016; Sultan *et al.*, 2016). Concerning *Eimeria* spp. prevalence, Chartier and Paraud (2012) assert that these protozoan species are more frequent in young animals, but in the present study the high prevalence was detected in adult sheep. Sheep Bc was correlated with the presence of *Eimeria* spp., which is understandable, as Chartier and Paraud (2012) have found that these parasites cause great economic losses because of diarrhoea.

Skrjabinema spp. is a rare parasite in sheep (Zajac & Conboy, 2012). Amarante (2014) described it in Brazil, where it has been

Table 2. Number and percentage of the different parasites found by municipality.

	Miranda do Douro		Vimioso		Mogadouro		Bragança			
	N = 357		*N* = 111		*N* = 34		*N* = 10		Total = 512	
Eggs/oocysts	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Strongyle-type	307	86.0	96	86.5	28	82.4	6	60.0	437	85.4
Nematodirus spp.	126	35.3	33	29.7	6	17.6	5	50.0	170	33.2
Moniezia benedeni	9	2.5	3	2.7	0	0.0	0	0.0	12	2.3
Moniezia expansa	4	1.1	0	0.0	0	0.0	0	0.0	4	0.8
Skrjabinema spp.	1	0.3	0	0.0	0	0.0	0	0.0	1	0.2
Trichuris spp.	24	6.7	7	6.3	1	2.9	1	10.0	33	6.4
Capillaria spp.	1	0.3	0	0.0	0	0.0	0	0.0	1	0.2
Dicrocoelium spp.	93	26.1	20	18.0	2	5.9	0	0.0	115	22.5
Fasciola hepatica	9	2.5	0	0.0	0	0.0	0	0.0	9	1.8
Eimeria spp.	262	73.4	94	84.7	23	67.6	7	70.0	388	75.8

Table 3. Percentage of low, moderate and high burden of infection, and mean, standard deviation and range of EPG/OPG.

	Burden of infection						
Parasite	Low	Moderate	High	Mean	SE	SD	Range
Strongyle-type	57.6	3.3	2.0	415.5	36.2	648.8	0–6250
Nematodirus spp.	8.6	0.0	0.0	90.9	13.4	89.1	0–550
Moniezia benedeni	1.4	0.0	0.0	150.0	37.8	100	0–350
Moniezia expansa	0.8	0.0	0.0	337.5	104.8	209.7	0–550
Trichuris spp.	0.6	0.0	0.0	50.0	0.0	0.0	0–50
Capillaria spp.	0.2	0.0	0.0	50.0	0.0	0.0	0–50
Eimeria spp.	56.8	5.9	5.7	670.1	75.9	1419.6	0–17550

SE, standard error; SD, standard deviation.

Table 4. Multivariate analysis of parasitic infections and potential risk factors.

	Strongyle-type eggs	*Nematodirus* spp.	*Eimeria* spp.	*Moniezia benedeni*	*Skrjabinema* spp.	*Dicrocoelium* spp.	*Trichuris* spp.
County	ns	ns	ns	ns	ns	ns	ns
Month	ns	ns	ns	5.25*	ns	34.07**	ns
Grazing	ns	4.66*	ns	ns	ns	11.11**	4.21*
Deworming	ns	4.51*	ns	ns	ns	ns	ns
Cohabitant animals							
Cattle	ns	ns	ns	ns	ns	ns	ns
Goats	ns	ns	ns	ns	ns	ns	ns
Donkeys	ns	ns	ns	ns	ns	ns	ns
Horses	ns	ns	ns	ns	ns	ns	ns
Dogs	ns	ns	14.83**	ns	ns	ns	ns
Bc	ns	ns	20.50**	ns	ns	ns	ns
Flock size	ns	ns	ns	ns	ns	ns	ns

**Significant for $P < 0.01$; *significant for $P < 0.05$; ns: non-significant ($P > 0.05$). Bc, body condition.

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Table 5. Results of linear regression analysis for strongyle, *Nematodirus* spp. and *Eimeria* spp. between deworming and body condition (Bc).

	Strongyle	*Nematodirus* spp.	*Eimeria* spp.
Factors			
Deworming	4.84*	6.04*	0.03^{ns}
Bc	13.48**	14.61**	10.24**
Number obs.	512	512	512
r^2	0.064	0.050	0.026

ns, non-significant ($P > 0.05$); *significant for $P < 0.05$; **significant for $P < 0.01$. Number obs., number of observations; r^2, coefficient of determination.

found in animals that share grass or cohabit with goats, as we confirmed to happen in this study. Moreover, to our knowledge, this is the first report of *Skrjabinema* spp. in Portugal.

The prevalence of *Trichuris* spp. was 6.4% and has been already reported in other European countries (Torina *et al.*, 2004; Pedreira *et al.*, 2006; Kantzoura, 2012). The prevalence and burden of this parasite is considerably higher than those found in neighbouring provinces in Spain, with the same or similar environmental conditions (Pedreira *et al.*, 2006).

The livestock husbandry systems and management practices can have a major influence on the transmission of that infection to a susceptible host population. Tariq *et al.* (2008) reported that faecal egg, pasture larval and worm counts were highest in communitary grazing sheep, as it still happens in this Portuguese region.

Two Cestoda species, *Moniezia expansa* and *M. benedeni*, commonly occur in ruminants (Strobel *et al.*, 2013; Diop *et al.*, 2015) and were found in 0.8% and 2.3%, respectively. While *M. expansa* was the most frequent Cestoda in some studies (Bashtar *et al.*, 2011; Nguyen *et al.*, 2012), worldwide, other researchers only report *M. benedeni* (Moazeni & Nili, 2004). October was significantly associated with increased prevalence of *M. benedeni*. This may be explained on the basis of parasites from the genera *Moniezia* being seasonal: peaks of infection occur in periods of greater activity of intermediate hosts, namely in spring and autumn (Taylor *et al.*, 2007; Bashtar *et al.*, 2011; Bowman, 2014).

The present study seems to suggest that the climate may have an impact in the occurrence of fasciolosis and dicrocoeliasis. *Fasciola hepatica* was only identified in Miranda do Douro, a municipality that falls within the catchment area of the River Douro, and flocks were located in villages with irrigated areas or near the Douro River, which is essential for the development of the intermediate hosts of *Fasciola* spp. Martínez-Valladares *et al.* (2013) have observed that *F. hepatica* occurrence was correlated significantly with the climate data.

The prevalence of *Dicrocoelium* spp. found is higher than that found in other European countries (Kantzoura, 2012; Taylor, 2012). Studies on geographic distribution and frequency rate of intermediate hosts are necessary for the control of the disease in animals and for the prevention of human infection. Those human infections are rare (Moure *et al.*, 2016), but can occur (Ella & Mohammad, 2015). As *Dicrocoelium* spp. and *Fasciola* spp. are zoonotic, sheep deworming is suggested. Sheep farmers and veterinarians need to be properly educated about the risks of zoonosis and about the importance of regular parasitological analysis.

FEC has been a diagnostic tool to evaluate infections, but has low sensitivity (Sargison, 2013; Atlija *et al.*, 2016). Mean EPG/OPG is commonly used as an indicator for the severity of an infection in a population. The degree of EPG/OPG in most samples was low, mirroring various reports in other regions worldwide (Tariq *et al.*, 2008; Idris *et al.*, 2012; Rinaldi *et al.*, 2015a; Atlija *et al.*, 2016) and in neighbouring regions in Spain with the same climatic conditions (Martínez-Valladares *et al.*, 2013).

The animal Bc was also related with the EPG mean of strongyle-type and *Nematodirus* spp. Van-Wik *et al.* (2006) report the same conclusion in their study with *H. contortus*.

The association observed between *Nematodirus* spp., *Trichuris* spp., *Dicrocoelium* spp. and grazing time was expected and is related to the epidemiology of these parasites (Bowman, 2014). In essence, it is important to limit the intake of infectious stages with pasture management, such as turn-out time and the duration of the grazing period (Thamsborg *et al.*, 2010). In relation to the *Dicrocoelium* spp. infection, it is important not to graze early in the morning or late in the afternoon, when there is the highest number of ants present on the herbage (Otranto & Traversa, 2003).

The severe infection found in one flock in this survey may be associated with the conditions of hygiene and over density observed. Deniz (2009) notes that coccidiosis in sheep is often associated with overcrowding and faecal contamination of drinking water or feed.

The fact that this study shows a low parasite burden in the CGM sheep breed can be explained by resistance and resilience to parasitism. Tariq *et al.* (2008) and McManus *et al.* (2014) reported with their surveys that the prevalence of GI parasites was higher in exotic breeds than in autochthonous ones.

In general, the overall prevalence of GI parasites in this study area still does not indicate GI parasites as a serious health problem; however, it is worth highlighting the importance of zoonotic parasites, especially among farmers and the human population. Effective veterinary care, routine epidemiological surveillance and enhanced educational campaigns on sheep parasitic zoonoses are key measures to increase productivity and reduce the public health risks associated with sheep farming in the north-east of Portugal.

The diversity of GI parasites and the consequences for sheep can become a serious threat to sheep production, especially in CGM sheep, an endangered autochthonous sheep breed. The constant surveillance and the ongoing refinement and development of parasite control strategies are crucial. These findings can be used to target high-risk farms with appropriate control measures against GI parasites of sheep in Portugal and other areas with similar climatic conditions. New studies should be conducted in the future to differentiate the GI nematodes involved, as well as *Eimeria* species.

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Conflicts of interest. None.

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