Epidemiological approach to nematode polyparasitism occurring in a sympatric wild ruminant multi-host scenario

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Abstract

The epidemiology behind multi-host/multi-parasite systems is particularly interesting to investigate for a better understanding of the complex dynamics naturally occurring in wildlife populations. We aimed to approach the naturally occurring polyparasitism of gastrointestinal nematodes in a sympatric wild ruminant scenario present in south-east Spain. To this end, the gastrointestinal tract of 252 wild ruminants of four different species (red deer, *Cervus elaphus*; mouflon, *Ovis aries musimon*; Iberian ibex, *Capra pyrenaica* and fallow deer, *Dama dama*) were studied in Cazorla, Segura y Las Villas Natural Park (Andalusia, Spain). Of the analysed animals, 81.52% were positive for parasite infection and a total of 29 nematode species were identified. Out of these, 25 species were detected in at least two host species and 11 parasitized all ruminant species surveyed. The multi-host interaction between these nematodes and the four host species is discussed under the perspective of host family-based differences.

Keywords: multi-host parasitism; polyparasitism; shared parasites; sympatry; wild ruminants

1. Introduction

Bronchopulmonary nematodes are widespread helminths found to parasite several free-ranging wild ungulates [1]. Their presence has a direct impact on domestic and wild ruminants, negatively affecting their health and fitness [2,3]. Bronchopulmonary infections usually course as subclinical diseases, although they have also been associated to respiratory disorders [1] and systemic signs such as weight loss or abortions [4,5]. Moreover, when bronchopulmonary nematode infection with microparasites or environmental stressors occurs, the course of the disease may progress to pneumonia [4].

Previous studies on the epidemiology of bronchopulmonary nematodes in wild ruminants have been carried out worldwide, including Spain [5–9]. Usually, hosts present co-infection with several lungworm species, and it has been shown that protostrongylid prevalence can be influenced by the interaction with other related lungworm species, such as *Dictyocaulus filaria* [10]. Interspecific parasite transmission between host ruminant species has also been described [11]. Examples of multi-parasite interactions in a complex wild host community have been documented in the literature for closely related ungulates, as showcased by a recent study in Southeast Spain that describes the gastrointestinal multi-host/multi-parasite system parasite richness occurring in sympatric wild ruminants [12]. The study of this interaction has proven itself particularly useful to study the role of pathogens influencing wildlife population dynamics [13–15]. However, no studies on lungworm community of sympatric ruminants in Spain have been carried out yet, and this is of particular interest considering the singular climatic conditions in southern Spain, as well as the diversity and abundance of these wild host populations there.

An optimal study area to investigate these dynamics is the “Sierras de Cazorla, Segura y Las Villas Natural Park” (SCSV), a hilly area of 2140 km^2 located on the eastern side of the Betic Mountains (Andalusia, Spain). In SCSVsizeable populations of four wild ruminant species can be found, including two species belonging to the Bovidae family (European mouflon *Ovis aries musimon* and Iberian ibex *Capra pyrenaica hispanica*) and two others which belong to the Cervidae family (red deer *Cervus elaphus* and fallow deer *Dama dama*) [16], providing this area with an interesting fauna to study natural lungworm infection. In this work, we aimed to investigate the epidemiological traits and ecology of the multi-host lungworm community using a multivariate abundance approach. This should highlight the risk factors associated with bronchopulmonary nematodes in a multi-host sympatric scenario.

2. Material and methods

2.1. Study area and wild ruminants

The study was carried out during the period 2003-2005 at SCSV. The Park has a continental Mediterranean mountain climate, and the annual rainfall ranges from 300 to 700 mm with a wet season in September and October. Large temperature variation is common, with an average annual temperature of 15°C [17]. A total of 250 wild ruminants of four different species were examined for lungworm presence: red deer, *n* = 64; Iberian Ibex, *n* = 19; mouflon, *n* = 59; fallow deer, *n* = 108. Sampling was limited to the hunting period (February to March). Age of animals was classified in three groups: group 1 (pre-adult animals, less than one year old), group 2 (young-adult animals, between one and two years old), and group 3 (adult individuals, older than two years old). Each animal was geolocalised in order to evaluate differences related to the sampling zone.

2.2. Sampling protocol

The respiratory tract, including lungs and trachea, was recovered and processed as described by Carrau et al. [18]. Briefly, lungs were cut up and 25 g were placed in gauze bags, using the Baermann-Wetzel method. First stage larvae (L1) were quantified in

Favatti counting chambers and expressed as L1 per lung gram (lpg). Larvae were identified to genus and, when possible, to species level according to Anderson et al., [19].

2.3. Epidemiological parameters and statistical analysis

Prevalence (the percentage of infected hosts with a particular parasite species / taxonomic group within the number of examined hosts), intensity (average number of individuals of a particular parasite species in a single infected host) and abundance (average number of individuals of a particular parasite species per host examined) for each lungworm species were defined according to Margolis et al. [20] and Bush et al. [21]. Fisher's exact test was used to evaluate the presence of significant differences among host species in prevalence data. A model-based analysis of multivariate abundance data, carried out using the mvabund package [22], was used to evaluate frequency distribution for the parasites shared by the four host species, and to evaluate the effect of risk factors such as host age and sex, and the sampling location. Kruskal–Wallis analysis was performed to statistically test the outcome. Analyses were carried out using R software [23].

3. Results

3.1. Overall descriptive patterns of the lungworms

A total of seven nematode genera were isolated, among which five species were identified: *Muellerius capillaris, Neostrongylus linearis, Protostrongylus* spp., *Dictyocaulus* spp, *Varestrongylus sagittatus, Cystocaulus ocreatus* and *Elaphostrongylus cervi*. All identified lungworms, as well as their respective prevalence, abundance and intensity are listed in Table 1.

Almost half (48.0%) of the animals were infected with bronchopulmonary nematodes. The mouflon was the host species with the highest prevalence (86.4%), followed by Iberian ibex (84.2%), red deer (56.3%) and fallow deer (15.7%). Significant differences in the prevalence of lungworms were detected among the four host species (Figure 2 and Table S1). In particular, lungworm prevalence in bovids (mouflon and Iberian ibex) was much higher than in cervids (red deer and fallow deer).

	Total		Iberian Ibex			Mouflon			Fallow deer			Red deer		
	P (%)	I.R.	P (%)	A	I.R.	P (%)	A	I.R.	P (%)	A	I.R.	P (%)	A	I.R.
Total	48.0	0 – 1896.0	84.2	6.59	0.1 – 35.5	86.4	106.2	0.1 – 189	15.7	2.0	0.1 – 0.6	56.3	3.0	0.1 – 49.5
C. ocreatus	22.0	0.1 – 1309.0	26.3	12.5	01 – 193.5	83.1	71.9	0.1 – 1309	--	--	--	--	--	--
Dictyocaulus spp.	4.4	0.1 – 111.7	0.0	0.0		15.3	2.5	0.1 – 111.7	1.8	0.1	0.1 – 1.3	0.0	0.0	0.0
E. cervi	14.4	0.1 – 49.5	--	--	--	--	--	--	--	--	--	56.3	0.3	0.1 – 49.5
M. capillaris	24.4	0.1 – 565.7	84.2	22.6	0.1 – 134.2	76.3	21.1	0.1 – 565.7	--	--	--	0.0	0.0	0.0
N. linearis	15.6	0.1 – 280.1	78.9	24.6	0.6 – 130.6	40.7	8.5	0.1 – 280.1	--	--	--	0.0	0.0	0.0
Protostrongylus spp.	9.2	0.1 – 65.2	57.9	6.1	0.5 – 65.2	20.3	2.2	0.1 – 56.4	--	--	--	0.0	0.0	0.0
V. sagittatus	6.0	0.1 – 6.2	--	--	--	--	--	--	13.9	0.2	0.1 – 6.2	0.0	0.0	0.0

Table 1: List of nematode species and their prevalence, abundance and intensity. P: Prevalence (%); A: Mean abundance (larvae per lung gram); I.R.: Intensity Range (minimum and maximum larvae per lung gram values).

3.2 Multi-host lungworm distribution

Two nematode genera (*Protostrongylus* spp. and *Dictyocaulus* spp.) and three species (*C. ocreatus, M. capillaris* and *N. linearis*) were found in at least two different host species. Most of these shared nematodes were found in bovids, while the red deer did not share any parasite species with the other hosts, as illustrated in Figure 1.

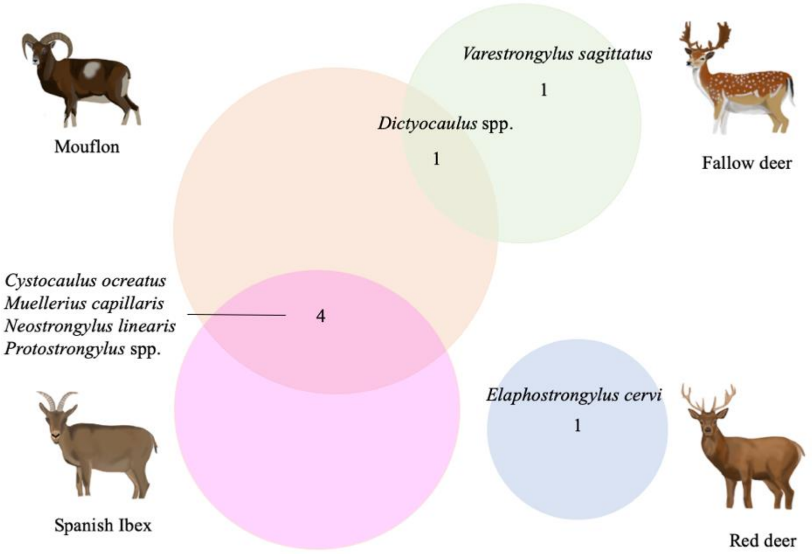

Figure 1. Venn diagram representing the number of lungworm species found in each species of wild ruminant host.

Lungworm prevalence showed differences between cervids and bovids (Fig. 2). The genus *Dictyocaulus* was identified in both host families, though with significantly different prevalence. However, mouflon and Iberian ibex shared up to four lungworm species, with significantly different prevalence in most scenarios. *Cystocaulus ocreatus* was predominantly found in mouflons, while *N. linearis* and the genus *Protostrongylus* were most commonly found in the Iberian ibex. Finally, *M. capillaris* was equally present in both bovids (Fig. 2).

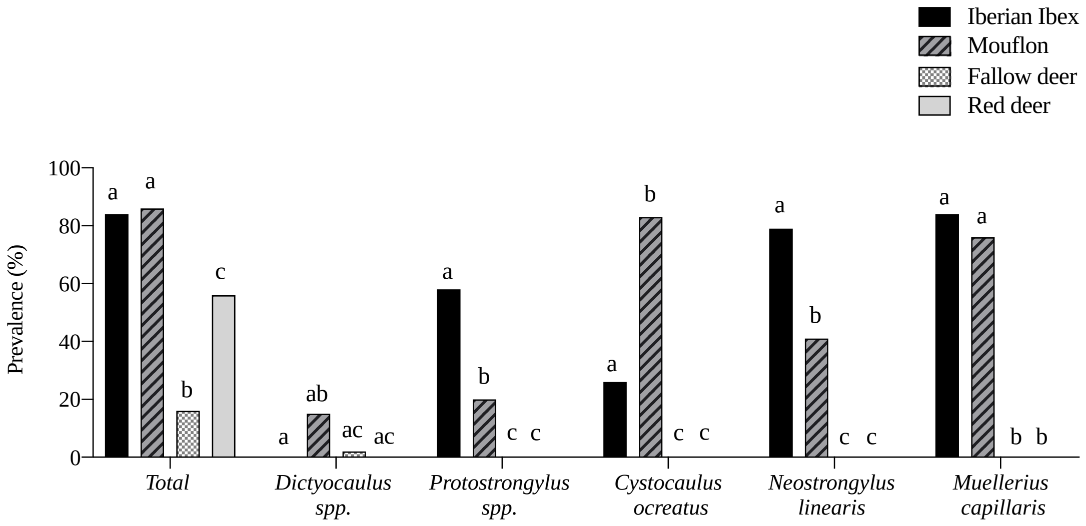

Figure 2. Total prevalence of bronchopulmonary nematodes and prevalence of the five commonly found genera and lungworm species. Different letters indicate significant differences between groups (*P* < 0.05).

Additionally, total mean abundance of the lungworm population showed significant differences amongst hosts (Fig. 3A - 3B). These differences held even at the parasite genus level between mouflon and fallow deer for *Dictyocaulus* (*P* = 0.0032). Likewise, abundances for *Protostrogylus* (*P* = 0.0025), *C. ocreatus* (*P* = 0.00018) and *N. linearis* (*P* = 0.0019) differed between both bovid hosts, with the exception of *M. capillaris* (*P* = 0. 38), which was found with similar abundance in mouflon and Iberian ibex (Table S2).

Finally, the implications of different factors on parasite abundance were also studied this group of sympatric populations. The multivariate abundance modeling approach highlighted a significant effect of host species and location on parasite abundance (*P* < 0.001), with the mouflon as the species with highest abundance and the central part of the Natural Park the area with most heavily infected animals (Figure 4). A marginal age effect was also detected (*P* < 0.1), with adult animals more likely to be heavily parasitized. Sex and year of sampling did not show any statistically significant differences in multi-host parasite abundance.

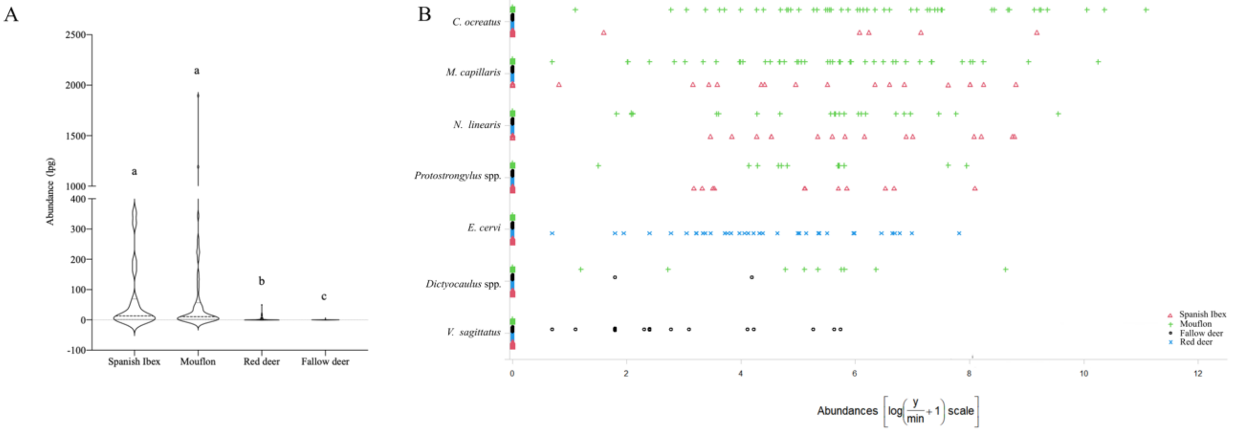

Figure 3. Abundance of total identified nematodes (A) and the abundance by parasite species (B). Different letters indicate significant differences between groups ($P < 0.05$).

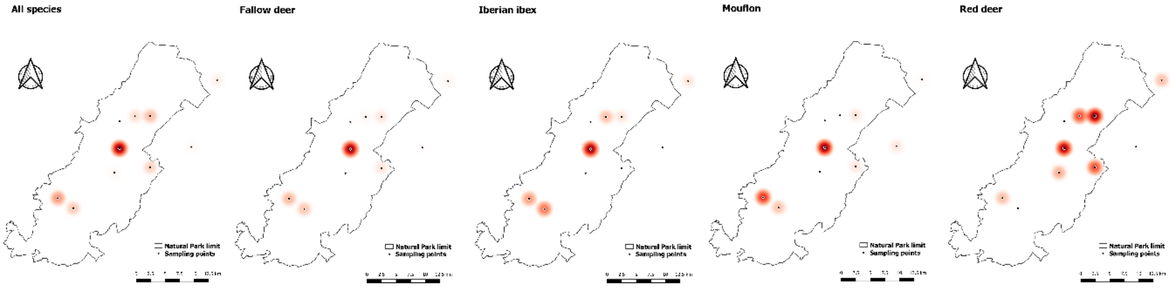

Figure 4. Spatial distribution of parasite abundance, considering all the host species together or each single wild ruminant species. A higher abundance corresponds to more intense red.

3.3 Single-host lungworms distribution

Elaphostrongylus cervi and *V. sagittatus* were found parasitizing only one host species; specifically, *E. cervi* was detected in the red deer population with a high prevalence (46.3%), while *V. sagittatus* (13.9%) was solely found in fallow deer.

4. Discussion

This study highlights the diversity of the bronchopulmonary nematode community shared amongst the wild ruminants in SCSV. A rich parasite community has been described, with seven different genera and/or species recorded in the sampled animals. Our results show that parasite richness is very similar between closely related ruminants, being richer in bovids than in cervids. Hence, this study represents an interesting example of parasite community structure and composition in a multi-parasite/multi-host scenario. Withn this interplay, the mouflon seems to be the epidemiological key in the present network of interactions, showing high prevalence and richness of lungworms that are shared with the other sympatric wild ruminant species. On the other hand, the red deer appears to be completely disconnected from the parasite community shared by the other three wild ruminant species present in SCSV, being infected only by *E. cervi*, a strictly species-specific parasite. Finally, the multivariate abundance analysis highlights that lungworm abundance at the community level is driven by host species identity, sampling location and, to a lesser extent, by age.

When compared with other wild ruminant populations, the recovered larvae species were in agreement with previous studies. The Iberian ibex is the most widely studied wild ruminant in Spain, and *M. capillaris* and *N. linearis* are the most prevalent lungworms found in this host species [24,25]. However, in contrast with the study carried out by Alasaad et al. [24], *Dictyocaulus filaria* was not present in our Iberian ibex population of SCSV. Similarly, previous studies conducted on mouflon describe a bronchopulmonary nematode community similar to that found in SCSV [26]. Cervids presented lower parasite richness, with the fallow deer as the most parasitized species in terms of the number of parasite species. *Varestrongylus sagittatus* has also been recorded in other European areas, although it should be noted that the prevalence we have found is the highest so far reported [27,28]. Finally, the prevalence of *E. cervi* found in red deer at SCVP was lower than in previous records in Spain [29]. Parasite richness in the different host species may be related to several factors, including host physiology and feeding behavior. Considering the life cycle of bronchopulmonary nematodes, the feeding behavior is directly modulating the risk of animals to enter into contact with the infective larvae. Under this perspective, it should be noted that the mouflon is the only of the four wild ruminant hosts classified as a grazer, while the other host species are classified as "intermediate" [30].

A multi-host/multi-parasite system was observed in three out of four host species, thus indicating a natural lungworm interchange between sympatric ruminants of SCSV. This phenomenon has been well described in other studies [11]. The parasite richness in SCSV was not related to the hosts' sex in the present work; however, there were differences across host species, sampling area and host age. The mouflon, from an epidemiological point of view, appears to be the key host species in the connection between cervids and bovids' lungworms. This wild ruminant was introduced in SCSV in 1953 and later in other areas of the Iberian Peninsula for hunting purposes, and adapted very well to these new habitats [31]. Mouflons play a major role in the maintenance and transmission of lungworms, as they share pastures in winter and spring with other wild bovids, such as the Iberian ibex [32], and in summer with wild cervids, such as fallow deer [33]. As described by Ezenwa [34], hosts that spend time in diverse habitats are more likely to acquire generalist parasites. In our previous study on the same sympatric wild ruminant populations, we already recorded multiple gastrointestinal parasite interchange between host species [12]. In this study, it was shown quite clearly that the mouflon may play a significant epidemiological role at the SCSV, as described for the gastrointestinal nematode community by Carrau et al. [12]. It is worth highlighting that the wild ruminant host community and its inner relationships in SCSV were deeply redesigned after the scabies outbreak that devastated the population of the Iberian ibex population [35]. A recent study comparing wild ruminant interactions before and after the Iberian ibex population crash demonstrated that the interaction among sympatric species is much more relevant now, with a higher possibility to exchange parasites [36].

High lungworm richness in wild ruminants has been shown to predict favorable climatic and ecologic conditions for the lungworm development cycle [37] as described by Alasaad et al. [24] in a similar sampling area. The dependence of bronchopulmonary nematodes on climatic conditions is a well established fact, even more so when gastropods are involved in their life cycles, as is the case for Protostrongylids [38–40]. According to Cabaret et al. (1994), L1 remain in the faeces and then eventually migrate onto the nearby vegetation if humidity allows it. The prevalence and intensity of infection of *N. linearis*, *M. capillaris* and *C. ocreatus* increases when relative humidity and rainfall increase, and decrease when the temperature decreases. Males are not included among the more parasitized animals even when the best climatic conditions for lungworms are registered; on the opposite, adult females seemed to shed more larvae coinciding with the periparturient period, which takes place during the months of higher temperature and humidity in our study. Similarly, Nocture et al. (1998) documented higher prevalences of *P. rupicaprae* and *N. linearis* in Alpine chamois (*Rupicapra rupicapra rupicapra*) coinciding with the periparturient physiological status of females. A possible influence of the climatic and environmental conditions on the parasite community can be seen by the presence of clusters in parasite abundance distribution, with the highest values localized in the central part of SCSV.

The results presented in this study represents an interesting pictures of the complex dynamics occurring at the pathogen-environmental-host interface, and can be used as a case study for the evaluation of similar scenario.

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Institutional Review Board Statement: The Ethical Committee for Animal Experimentation of the University of Murcia reports that, following the basic rules applicable for the protection of animals used in experimentation and other scientific purposes (described in RD 53/2013), procedures in this study are considered to be out of the scope of application of said RD since we do not use live animals, but carcasses donated from authorized hunts in the study area.

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Table S1. Pairwise comparisons of the wild ruminants prevalence using Fisher's exact test.

Total lungworms

	Iberian Ibex	Red deer	Fallow deer
Red deer	3.851e-02	-	-
Fallow deer	2.593e-08	1.038e-07	-
Mouflon	1.000	4.509e-04	5.805e-19

C. ocreatus

	Iberian Ibex	Red deer	Fallow deer
Red deer	4.806e-04	-	-
Fallow deer	6.861e-05	1.000	-
Mouflon	1.977e-05	3.122e-24	7.144e-32

M. capillaris

	Iberian Ibex	Red deer	Fallow deer
Red deer	2.811e-14	-	-
Fallow deer	2.373e-17	1.000	-

Mouflon	6.508e-01	4.449e-21	6.269e-28

Protostrongylus spp.

	Iberian Ibex	Red deer	Fallow deer
Red deer	1.405e-08	-	-
Fallow deer	2.039e-10	1.0000000	-
Mouflon	4.000e-03	0.0001167	3.416e-06

Dictyocaulus spp.

	Iberian Ibex	Red deer	Fallow deer
Red deer	1.0000	-	-
Fallow deer	1.0000	0.79498	-
Mouflon	0.2065	0.00474	0.00474

N. linearis

	Iberian Ibex	Red deer	Fallow deer
Red deer	6.881e-13	-	-
Fallow deer	1.993e-15	1.000e+00	-
Mouflon	8.770e-03	1.547e-09	6.881e-13

Varestrongylus sagittatus

	Iberian Ibex	Red deer	Fallow deer
Red deer	1.0000	-	-
Fallow deer	0.2489	0.003863	-
Mouflon	1.0000	1.000000	0.003863

Elaphostrongylus cervi

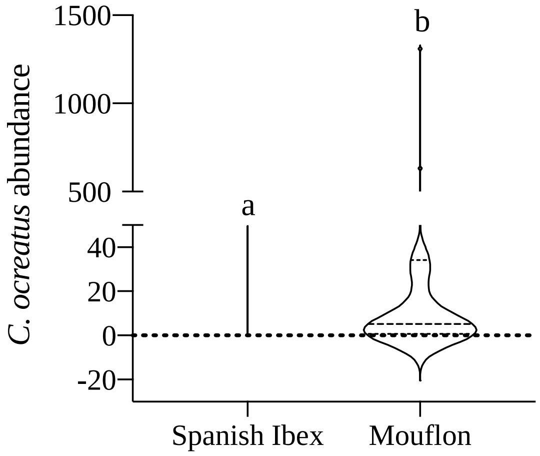
	Iberian Ibex	Red deer	Fallow deer
Red deer	5.796e-06	-	-
Fallow deer	1.000e+00	4.280e-19	-
Mouflon	1.000e+00	2.195e-13	1

Table S2. Kruskal–Wallis analysis for the commonly found nematodes species.

C. ocreatus

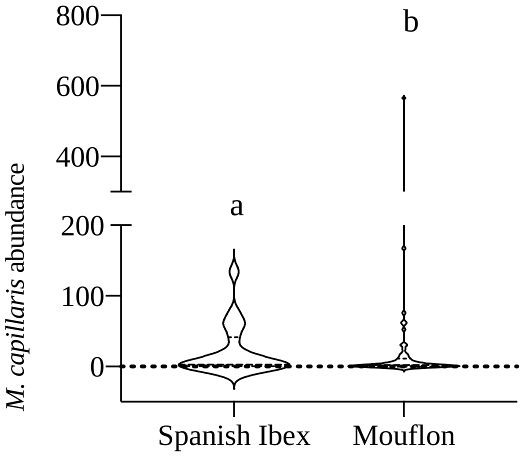
	Iberian Ibex	Red deer	Fallow deer

Red deer	3.5e-05	-	-
Fallow deer	1.1e-07	-	-
Mouflon	0.00018	< 2e-16	< 2e-16



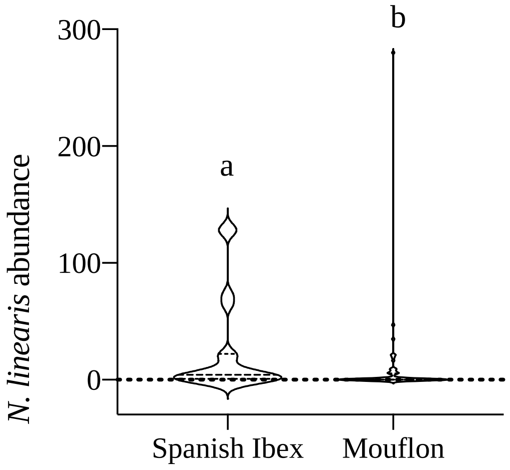
M. capillaris

	Iberian Ibex	Red deer	Fallow deer
Red deer	1e-15	-	-
Fallow deer	<2e-16	-	-
Mouflon	0.38	<2e-16	<2e-16



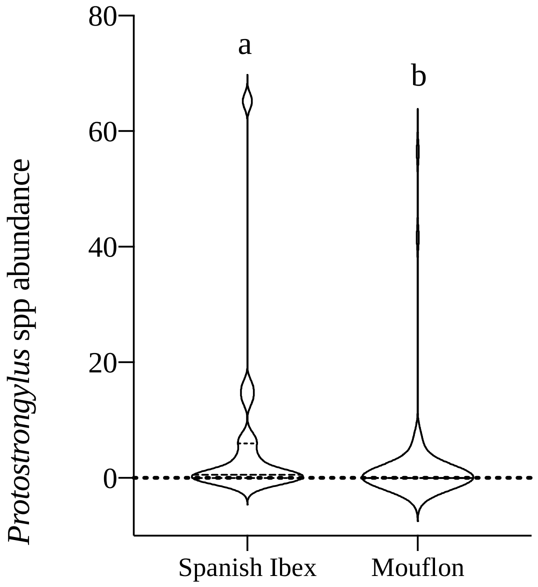
N. linearis

	Iberian Ibex	Red deer	Fallow deer
Red deer	2.4e-14	-	-
Fallow deer	< 2e-16	-	-
Mouflon	0.0019	2.4e-08	1.9e-12



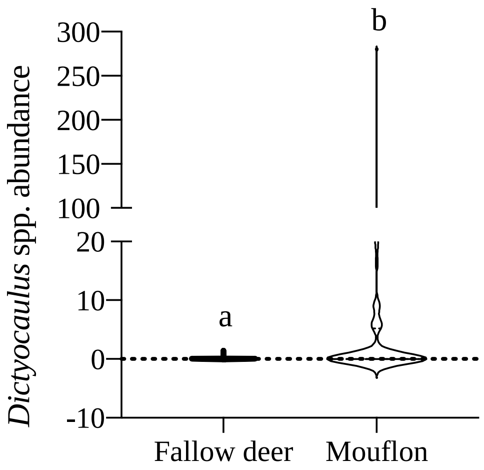
Protostrongylus spp.

	Iberian Ibex	Red deer	Fallow deer
Red deer	2.5e-10	-	-
Fallow deer	9.9e-16	-	-
Mouflon	0.0025	0.0002	2.1e-06



Dictyocaulus spp.

	Iberian Ibex	Red deer	Fallow deer
Red deer	-	-	-
Fallow deer	0.5620	0.3483	-
Mouflon	0.1242	0.0032	0.0032


Dictyocaulus spp. abundance
300
250
200
150
100
20
10
0
-10
b
a
Fallow deer
Mouflon