Journal of Helminthology (2005) **79**, 113–118 DOI: 10.1079/JOH2005285

Relationship between bronchopulmonary nematode larvae and relative abundances of Spanish ibex (*Capra pyrenaica hispanica*) from Castilla-La Mancha, Spain

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Abstract

The excretion of bronchopulmonary nematode infective larvae was evaluated in 160 faecal samples of Spanish ibex (*Capra pyrenaica hispanica*) collected from 13 populations in Castilla-La Mancha, south-central Spain in September 2003. Intensities and prevalences were compared with pasture availability, abundances of wild and domestic ungulates at both levels, i.e. for populations and for faeces in a two-step procedure. Protostrongylid larvae showed similar infection rates (mean intensity: 1.56 ± 0.12, $n = 94$; mean prevalence: $25.62 \pm 6.86\%$, $n = 160$) to *Dictyocaulus* spp. (mean intensity: 1.03 ± 0.11, $n = 48$; mean prevalence: $30.00 \pm 7.11\%$, $n = 160$). At the population level, positive correlations were found between the prevalences of both bronchopulmonary taxa. The prevalence in both groups, but not intensity, also correlated positively with Spanish ibex abundance indexes both for the populations and individual faeces. These findings suggest that: (i) parasite spreading across Spanish ibex populations in Castilla-La Mancha could respond to host density-dependent processes; and (ii) these populations may have similar exposition and/or susceptibility to both bronchopulmonary taxa resulting in similar host–parasite patterns, despite their different life cycles. Bronchopulmonary outputs in the Spanish ibex from Castilla-La Mancha seems not to represent a health risk for this endemic wild ungulate but may be useful in any health surveillance scheme for the increasing populations of Spanish ibex.

Introduction

Bronchopulmonary nematode parasites are frequently found parasitizing wild ungulates. These include several homoxenous species belonging to the family Dictyocaulidae (*Dictyocaulus* spp.), and heteroxenous parasites of the family Protostrongylidae (*Cystocaulus* spp., *Neostrongylus* spp., *Muellerius* spp., *Protostrongylus* spp.), which parasitize terrestrial gastropods as secondary hosts (Anderson, 2000). Infections are commonly asymptomatic, but they may occasionally cause severe verminous bronchopulmonary pneumonia and nodular lesions in the lungs (Boch & Schneidawind, 1988). Usually, fatal cases are associated with concomitant bacterial infections, and predisposing factors, such as increased exposition to infective larvae in captivity, are involved (Charleston, 1980). Infections by lungworms in wild ungulates warrant the attention of researchers and wildlife managers (e.g. Festa-Bianchet, 1989; Forrester & Lankester, 1997; Enk *et al.*, 2001; Vicente & Gortazar, 2001).

The study of infective larval stages in faeces has been employed as a non-invasive alternative technique to study host–parasite relationships in wild ungulate populations (e.g. Festa-Bianchet, 1991). Both population and individual factors affecting bronchopulmonary adult infection or larval outputs have been evaluated in wild ungulates (e.g. Hugonnet & Cabaret, 1987; Arnett *et al.*, 1993), but there is little published on mountain ungulates (Manfredi *et al.*, 1996).

The Spanish ibex (*Capra pyrenaica hispanica*) is a medium-sized mountain ungulate endemic to the Iberian Peninsula. It is mainly distributed through most of the

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Mediterranean range mountains and, to a lesser extent, in central and northern Spain (Pérez *et al.*, 2002). Currently, the populations at Castilla-La Mancha are increasing both in distribution area and densities (Acevedo *et al.*, 2003). Thus, health parameters of this species need to be monitored in addition to ecological and demographic studies of the Spanish ibex, other sympatric wildlife, and domestic livestock.

In this context, the objective was to assess at the population level the relationships of Dictyocaulidae and Protostrongylidae bronchopulmonary faecal larvae with the abundance indexes of the Spanish ibex and sympatric wild and domestic ungulates in 13 populations from Castilla-La Mancha.

Materials and methods

Fresh faecal samples (n = 160) were collected in conjunction with an ecological programme on Spanish ibex relative abundances in 13 areas (fig. 1) throughout the current distribution of the species in Castilla-La Mancha (UTM coordinates: 30S 294,348-681,063 4,208, 706-4,575,340; minimum altitude = 244 m, maximum altitude = 2274 m) during September 2003. Since ungulate droppings tend to be aggregated, we developed a simple method using dropping frequency (instead of their number) to estimate the relative abundance of wild ungulates including Spanish ibex and red deer (*Cervus elaphus*) (Vicente *et al.*, 2004). Briefly, each count consisted of n = 30 transects of 100 m, divided into ten sectors of 10 m in length. The dropping frequency was defined as the average of the number of 10 m sectors with Spanish ibex or red deer droppings in each transect of 100 m. ($DF = \Sigma Di/n$; where D is the number of dropping-positive sectors and ranges from 0 to 10, and n is the number of 100 m transects, usually 30). Habitat and management variables considered in the study were chosen on the basis of their epidemiological significance to survival and transmission of infective stages, and the characteristics of local big game and livestock. Environmental conditions for the 13 study areas were recorded every 200 m in 30 buffer areas of 25 m radius across the linear transects (n = 15 points per area) to finally obtain mean values of habitat land uses and habitat structure of each estate. Thus, we considered shrub/forest/grass/soil covers (%) and habitat availability (Mediterranean shrublands, Dehesa (Mediterranean savannah-like habitat), Mediterranean hardwood forest, (*Quercus* spp.), pine plantations, pastures, riparian habitat, agricultural areas). Data from private and government gamekeepers regarding the number of livestock in each study area were obtained through personal interviews.

Bronchopulmonary larvae were extracted from 5 g of faeces using the beaker larval migration technique described by Forrester & Lankester (1997). Larvae were quantified in a Favatti counting chamber and identified to the genus level according to their morphology and linear lengths using a calibrated eyepiece micrometer (Melhorn *et al.*, 1992; Cordero del Campillo & Rojo, 1999; Anderson, 2000).

The terms relating to prevalence and intensity of infection are as described by Margolis *et al.* (1982). Standard errors for prevalences were estimated with the

Fig. 1. The location of sampling areas where faeces of Spanish ibex were collected within Castilla-La Mancha, during September 2003.

https://doi.org/10.1079/JOH2005285 Published online by Cambridge University Press

expression S.E. (p) = p(1-p)/n1/2 (Martin *et al.*, 1987). Analyses of factors affecting the prevalence and intensity of bronchopulmonary larvae were performed at two levels. First, the bivariable association of mean excretion rates (prevalences and intensities) at the population level for overall bronchopulmonary parasites, Dictyocaulidae, overall Protostrongylidae larvae and the different genera were compared with habitat variability (pasture covering) and other ungulate (cattle, sheep/goat or deer) abundance indexes using non-parametric Spearman rank-correlation coefficients (r_s, n = 13). Mann-Whitney and Chi^2 non-parametric tests were employed to compare the intensities and prevalences of the different protostrongylid genera.

To clarify the initial findings at the population level to elucidate the relative importance of explanatory variables (avoiding the multiple test problem), the outcome variables from the previous analysis were evaluated by means of multivariate analysis for overall bronchopulmonary, Dictyocaulidae and overall Protostrongylidae throughout the faeces (n = 160). Generalized linear models (GLM) were conducted with the overall bronchopulmonary, Dictyocaulidae and overall Protostrongylidae larvae as response variables, respectively (considered as binomial: presence, 1; or absence, 0). To ensure that relationships were not driven by a few points of small sample size, the sample size at the population level was controlled by including this in the model as an explanatory variable. Habitat (pasture availability) was also included as an explanatory variable. Finally, in the case of the Dictyocaulidae and Protostrongylidae models, both the response and explanatory variables concerning larval presence (binomial variables: presence, 1; or absence, 0) respectively were interchanged to identify any significant relationships between these taxa for faeces. A negative binomial error distribution and a logistic link function (Crawley, 1993) were considered. The resulting models were reduced to their simplest form by eliminating, in a backward stepwise manner, any explanatory variables that failed to explain significant variation in the response. The level of significance was established at the 5% level. All *P*-values refer to two-tailed tests, using SPSS 10.0.6 program (SPSS Inc., 1999) and Statistica (Statsoft Inc., 1999) software packages.

Results

Infection rates of bronchopulmonary larvae in Spanish ibex faeces from Castilla-La Mancha are shown in table 1 at the population level. Both overall Protostrongylidae (mean intensity = 1.56 ± 0.12, n = 94; mean prevalence: 25.62 ± 5.88%, n = 160) and *Dictyocaulus* spp. (mean intensity = 1.03 ± 0.11, n = 48; mean prevalence = 30.00 ± 43.14%, n = 160) larvae in faeces showed similar rates. Prevalences of Protostrongylidae and *Dictyocaulus* spp. correlated positively and significantly at the population level ($P < 0.05$, fig. 2). The prevalences of overall Protostrongylidae and *Dictyocaulus* spp. correlated positively with the Spanish ibex relative abundance indexes (fig. 3). Regarding the effects of habitat on parasites, pasture availability correlated positively with the mean prevalence of *Protostrongylus* spp. ($P < 0.05$).

From the analysis of individual faeces, the presence of overall bronchopulmonary, Protostrongylidae and *Dictyocaulus* spp. larvae in faeces showed an association with higher Spanish ibex abundance indexes ($P < 0.001$, $P < 0.01$ and $P < 0.01$ for overall bronchopulmonary, Protostrongylidae and *Dictyocaulus* spp. larvae models, respectively). In both the Protostrongylidae and *Dictyocaulus* spp. models there was no evidence to link faeces infected with *Dictyocaulus* spp. with protostrongylid infections and vice versa.

Four different genera of Protostrongylidae were identified: *Cystocaulus* spp. (mean intensity = 1.51 ± 0.22, n = 13; mean prevalence = 8.12 ± 4.25, n = 160); *Neostrongylus* spp. (mean intensity = 1.62 ± 0.21, n = 32; mean prevalence = 20.00 ± 6.22, n = 160), *Muellerius* spp. (mean intensity = 1.74 ± 1.17, n = 38; mean prevalence = 23.75 ± 6.61, n = 160) and *Protostrongylus*

Table 1. Mean prevalence ± $S.E._{95\%\ I.C.}$ and mean intensity of infection of the Spanish ibex with Protostrongylidae and *Dictyocaulus* spp. larvae.

	Protostrongylidae		*Dictyocaulus* spp.	
Sampling area	Mean intensity ± $S.E._{95\%\ I.C.}$	Mean prevalence ± $S.E._{95\%\ I.C.}$	Mean intensity ± $S.E._{95\%\ I.C.}$	Mean prevalence ± $S.E._{95\%\ I.C}$
Madrona (30)	1.64 ± 0.35 (14)	46.67 ± 18.15	1.52 ± 0.48 (5)	16.67 ± 13.57
Viso Marqués (5)	1.75 ± 0.00 (1)	20.00 ± 39.20	0	0.00 ± 0.00
Garganta (10)	1.20 ± 0.21 (8)	80.00 ± 26.13	0.91 ± 0.22 (5)	50.00 ± 32.67
Becerras (10)	1.62 ± 0.43 (7)	70.00 ± 29.95	1.32 ± 0.37 (3)	30.00 ± 29.95
Fuertescusa (10)	1.68 ± 0.38 (5)	50.00 ± 32.68	0.85 ± 0.00 (1)	10.00 ± 19.60
S. Cuenca (13)	1.47 ± 0.62 (5)	45.50 ± 30.89	1.36 ± 0.20 (4)	30.80 ± 26.07
Cabriel (10)	1.45 ± 0.46 (5)	50.00 ± 32.67	0	0.00 ± 0.00
Liétor (10)	1.29 ± 0.39 (8)	80.00 ± 26.13	0.79 ± 0.32 (4)	40.00 ± 32.01
Riopar (12)	0.93 ± 0.38 (5)	41.67 ± 29.13	0.66 ± 0.099 (4)	33.33 ± 27.85
Salobre (10)	2.17 ± 0.17 (6)	60.00 ± 32.01	1.16 ± 0.35 (5)	50.00 ± 32.67
Bogarra (21)	1.17 ± 0.19 (17)	80.95 ± 17.21	0.89 ± 0.20 (12)	57.14 ± 21.70
Casas Lázaro (8)	2.18 ± 0.36 (3)	37.50 ± 35.87	1.08 ± 0.00 (1)	12.50 ± 24.50
Yeste (11)	2.40 ± 0.18 (10)	90.91 ± 17.82	0.91 ± 0.18 (4)	36.36 ± 29.81
Overall	1.56 ± 0.12 (94)	25.62 ± 5.88	1.03 ± 0.11 (48)	30.00 ± 43.14

Numbers in parentheses indicate sample size of faecal samples examined. Standard error of the prevalence is referred as S.E.

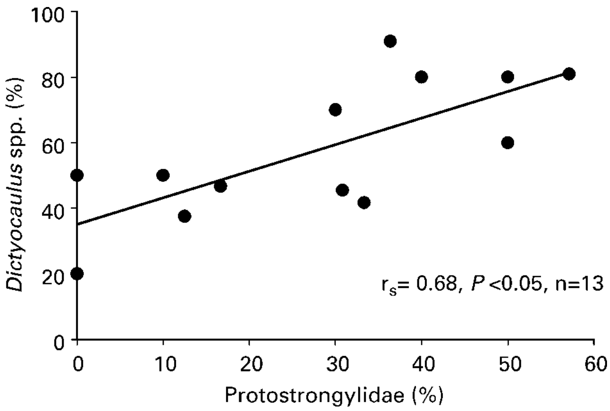

Fig. 2. The relationship between the prevalences (%) of Protostrongylidae and *Dictyocaulus* spp. larvae in the Spanish ibex from 13 sampling sites in Castilla-La Mancha, Spain.

spp. (mean intensity = 1.17 ± 0.15, n = 19; mean prevalence = 11.87 ± 6.05, n = 160). Thus, all showed similar intensity rates ($P > 0.05$ referred to all comparisons of pairs), but *Muellerius* spp. and *Neostrongylus* spp. showed higher prevalence values than *Cystocaulus* spp. and *Protostrongylus* spp. ($P < 0.05$ referred to comparisons of pairs). When considering the different genera of Protostrongylidae at the population level, *Cystocaulus* spp. correlated positively with the abundance index of small domestic ruminants (including sheep and goats) ($P < 0.01$) and with the density of small ruminants (estimated as heads per km^2) according to keeper interviews ($P < 0.05$).

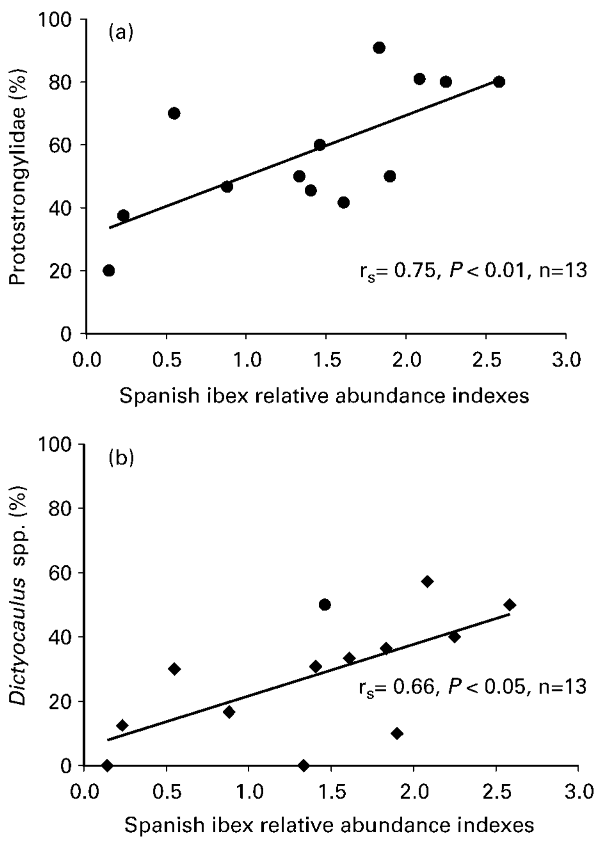

Fig. 3. The relationship between the prevalences (%) of (a) Protostrongylidae and (b) *Dictyocaulus* spp. larvae and relative abundance indexes of the Spanish ibex in 13 sampling sites in Castilla-La Mancha, Spain.

Discussion

All taxa of bronchopulmonary parasites found in the present study had previously been reported in the Spanish ibex. The little available literature comes from Andalusia (southern Spain), and includes either necropsies (Montero *et al.*, 1998, in Sierra Nevada), or both necropsies and coprological examinations (Universidad de Jaén 1999, in several mountainous areas from Andalusia). This lack of information limits the comparisons between the present infection rates and previous data.

As in the present study, *Muellerius* spp. (mean prevalence: 25.77%) was also the most common protostrongylid species in the previous literature, with a prevalence of 74.30%, always in concomitant infections with other bronchopulmonary parasites (Universidad de Jaén, 1999). To date, the only species of the genus *Muellerius* spp. described in the Spanish ibex is *Muellerius capillaris* (Montero *et al.*, 1998; Universidad de Jaén, 1999). Our overall prevalences, not only for *Muellerius* spp., but also for the remainder of the protostrongylids, are lower than those previously described in coprosurveys. Regarding *Dictyocaulus* spp., the overall prevalence data (26.35%) shows a higher prevalence than previously reported in coprological analyses of 1.20% (Universidad de Jaén, 1999) but lower than that reported at necropsy by Montero *et al.* (1998) (37.5%). In addition to the scarcity of data, the present data refer to late summer, and consequently are not directly comparable to those previously reported, as lower values in prevalence have been found in the summer and autumn as compared to winter and spring in the Spanish ibex (Universidad de Jaén, 1999) and alpine ibex (*Capra ibex ibex*, Manfredi *et al.*, 1996). Despite this, *Dictyocaulus* spp. presents a higher prevalence when compared with the majority of the Andalusian populations (Universidad de Jaén, 1999). Apart from differences in seasonal sampling, a beaker extraction method as described by Forrester & Lankester (1997) was used in the present study, which is an improvement on the classical Baerman funnel method, but makes comparisons between past and present data difficult.

Factors such as exposure and/or differences in susceptibility to parasites could be operating at the population level, and hence differences between host and parasite populations could occur (Wilson *et al.*, 2001). In particular, there was a correlation between the prevalences of both groups of bronchopulmonary parasites (Protostrongylidae and *Dictyocaulus* spp.) and host abundances. However, with field collected faeces, host sample size is unknown but in the present study a positive association between the prevalence of the parasite taxa (overall bronchopulmonary, *Dictyocaulus*

https://doi.org/10.1079/JOH2005285 Published online by Cambridge University Press

spp. and protostrongylids) and host abundance supports the idea of density-dependent processes operating in the transmission of bronchopulmonary nematodes in the Spanish ibex. This is consistent with epidemiological models which predict a positive relationship between host population density and abundance of macroparasites since the transmission rate generally is a positive function of host population density (Arneberg *et al.*, 1998; Arneberg, 2001). This relationship was noted for both indirectly (protostrongylids) and directly transmitted (*Dictyocaulus* spp.) nematodes. However, the limited sample sizes of this scarce wild ungulate in Castilla-La Mancha, which constitutes populations at their distribution limit coming from other regions, must be taken into account before precise conclusion can be drawn. More research is needed to elucidate this aspect.

The Spanish ibex presents a wide range of densities in Castilla-La Mancha (fig. 3), ranging from 0.2 to 5.8 animals per km^2, which may be due to the current spatial expansion of this species in Castilla-La Mancha, offering different epidemiological scenarios to parasite transmission. The parasite–host density relationships are mainly related to parasite dissemination across the different host populations, but not to intensities. The effects of parasites on hosts are expected to be parasite load dependent (e.g. Albon *et al.*, 2002), and suggest that current bronchopulmonary infection loads are not causing any detrimental effects on the studied populations, since larval counts were not high enough to produce clinical infections (Cordero del Campillo & Rojo, 1999).

With reference to the relationship between habitat and parasites, a correlation between pasture availability and the prevalence of *Protostrongylus* spp. was demonstrated. Since pastures are a limited resource in Mediterranean habitats, this finding could be related to transmission rates of the parasite depending on the pasture microhabitat availability for infective larvae and intermediate hosts (gastropods), and on host aggregation in these areas. An additional explanation may be related to pastures shared between Spanish ibex and domestic livestock. Only the prevalence of *Cystocaulus* spp. was correlated with livestock abundance, supporting the idea that wild and domestic ruminants share at least some parasite genera, which eventually could be transmitted at the interface.

The present study has therefore indicated a relationship between the abundance of a wild ungulate, the Spanish ibex, and the prevalence of bronchopulmonary parasites, suggesting density-dependent transmission rates of these parasites. Therefore, monitoring herd levels for evidence of bronchopulmonary parasite infections could prove to be a valuable tool for wildlife health managers in the surveillance of populations of the endemic Spanish ibex.

Acknowledgements

We are very grateful to public managers and gamekeepers for their help in the fieldwork. This is a contribution to Junta de Comunidades de Castilla-La Mancha (Convenio CAMA). Joaquín Vicente was supported by a predoctoral grant from the Junta de Comunidades de Castilla-La Mancha and Vanesa Alzaga had a partial grant from Universidad de Castilla-La Mancha.

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(Accepted 3 February 2005)