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ORIGINAL PAPER

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Epidemiology and risk factors analysis of elaphostrongylosis in red deer (*Cervus elaphus*) from Spain

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Abstract We studied the distribution and faecal shedding pattern of the first-stage larvae (L1) of *Elaphostrongylus cervi* (Nematoda: Protostrongylidae) in the red deer (*Cervus elaphus*) across Spain, where excretion was widespread. We evaluated the effects of individual, population and environmental factors on *E. cervi* L1 counts in 18 free-ranging red deer populations in South Central Spain. In this area, prevalence was 71.42±2.14% (n=448) and mean intensity (n=320) was 74.50±10.35. Aggregation of deer at water-holes was positively associated with *E. cervi* L1 prevalence, possibly due to spatial and temporal odds of infected gastropods, red deer and infective *E. cervi* L1 larvae being encountered. Prevalence increased with age, and there was also a trend towards males having higher intensities than females. A slightly decreasing age–intensity profile was identified for females, which may suggest a role of acquired immunity.

Introduction

Elaphostrongylus cervi Cameron 1931 (Nematoda: Metastrongyloidea) are parasites of the central nervous system and skeletal muscles of red deer, *Cervus elaphus* (Mason 1995; Lankester 2001). The parasite has a wide natural distribution in Eurasia but is also present in New Zealand. The presence of large spiny-tailed protostrongylid larvae similar to those of *E. cervi* and immature *E. cervi* adults has also recently been reported for Central and South Spain (Vicente and Gortazar 2001; Valcárcel and Romero 2002).

Red deer acquire infection by accidentally ingesting gastropod intermediate host containing infective third-stage (L3) larvae of the nematode. These are liberated in the deer gut and mature during migration. *E. cervi* reach the adult stage in the CNS (subarachnoid spaces) and subsequently migrate into the fascia and connective tissue around skeletal muscles, where they mature and live in reproductive pairs and groups (Handeland et al. 2000). Presumably, adult females lay eggs that by the haematogenous route reach the lungs, where they hatch as first-stage larvae (L1). These travel up the bronchial tree, are swallowed and are dispersed in the host faeces. In the environment, L1 penetrate the foot of terrestrial gastropods, where they develop to the infective L3 (Rezac et al. 1994). Although clinical signs due to *E. cervi* infection are uncommon in red deer and elk (Watson 1983, but see Handeland et al. 2000), neurological signs and deaths result from natural or experimental infection of other Cervidae (Gajadhar and Tessaro 1995) as well as in domestic goats (Handeland et al. 2000).

In Spain, several wild ungulates including the deer have expanded their range in the last decades (Gortazar et al. 2000). Changes in wildlife management have already raised concerns regarding the control of infectious and parasitic diseases (e.g. Vicente et al. 2004a). Since *E. cervi* has considerable pathological consequences for other wild and domestic ungulates (Gajadhar and Tessaro 1995), it is important for wildlife managers, livestock breeders and sanitary authorities to know its abundance and distribution, especially when concurrent wild ungulate species co-occur with valued species such as the Spanish Ibex (*Capra pyrenaica*). Prevalence and intensity of infection and the dynamics of transmission may vary according to the availability of hosts and to local environmental conditions (Njau et al. 1992). In contrast with other Elaphostrongylinae worm/deer systems (e.g. Wasel et al. 2003), little is known regarding the correlation of ecological and management risk factors with infection rates of *E. cervi* in red deer populations. In this context, our objectives were to extend the knowledge of the distribution of *E. cervi* across Spanish Iberian red deer populations and to evaluate individual, population and environmental risk factors on *E. cervi* infection through the evaluation of L1 faecal counts.

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Material and methods

Study sites

A total of 46 red deer populations (n=970 individuals) from throughout Spain were sampled to determine the general distribution of the parasite in the country (Figs. 1 and 2). This included samples from Northern Atlantic Spain (Cantabric and Pyrinean Mountains, respectively), Northern Central Spain (the Iberian mountains), South Central Spain (Montes de Toledo, Sierra Morena and Betic chains mountains) and finally in South Spain (Los Alcornocales Natural Park).

A variety of hunting estates are found across South Central Spain supporting what can be classified as semi-free-ranging cervids (large enclosures with relatively low densities of deer) and free-ranging cervids (not enclosed or intensively managed). We evaluated the effect of potential risk factors on *E. cervi* prevalence and intensity across 18 populations of red deer (Table 1) from Ciudad Real province and boundaries, South Central Spain (37°13′48″N to 39°31′43″N in latitude and from 06°34′06″W to 2°25′54″W in longitude, Figs. 1 and 2). The habitat in this area is Mediterranean and characterized by *Quercus ilex* forest and scrublands (dominated by *Cystus* spp, *Pistacia* spp, *Rosmarinus* spp, *Erica* spp and *Phyllirea* spp), with scattered pastures and small areas with crops. Seasonal streams cross many of the estates, but water is retained and available all-year-round in water-holes, in most cases artificially made along riverbeds. This area includes Montes de Toledo and Sierra Morena mountains. The Guadiana River valley consists of fragmented Mediterranean habitat and is a transition between Montes de Toledo and Sierra Morena (Fig. 2). The climate is Mediterranean. Annual rainfall is highly variable and ranges from 300 to 700 mm. The wet season typically extends from October to May when most of yearly rains are received. The dry season (from June to September) is when food and water resources become limited for ungulates. We deliberately chose sites where a priori a range of densities and management factors would be present. We rejected from the risk analysis hunting estates where anthelminthic treatment (usually orally on food) was supplied.

Sampling and diagnosis

Data were collected during the hunting season in a cross-sectional nationwide survey of wild red deer in Spain from year 2000 to 2004. Fresh faecal samples were collected from hunted animals directly from the rectum during field necropsy. Protostrongylid larvae were extracted in less than 24 h from 8 to 10 g of faeces using the Baermann beaker extraction method described by Forrester and Lankester (1997). Larvae were quantified in a Favatti counting chamber and expressed as number of L1 per gram of wet faeces (lpg). Larvae were identified to genus level according to their morphology and linear dimensions using the descriptions in Kutzer and Prosl (1975), English et al. (1985), Demiaszkiewicz (1986), Rezac (1990) Mason (1995), Vicente and Gortazar 2001 and Lankester (2001). The morphology and size of the dorsal-spined larvae were similar to the first-stage larvae of Elaphostrongylinae and not with those from other protostrongylids with spiny-

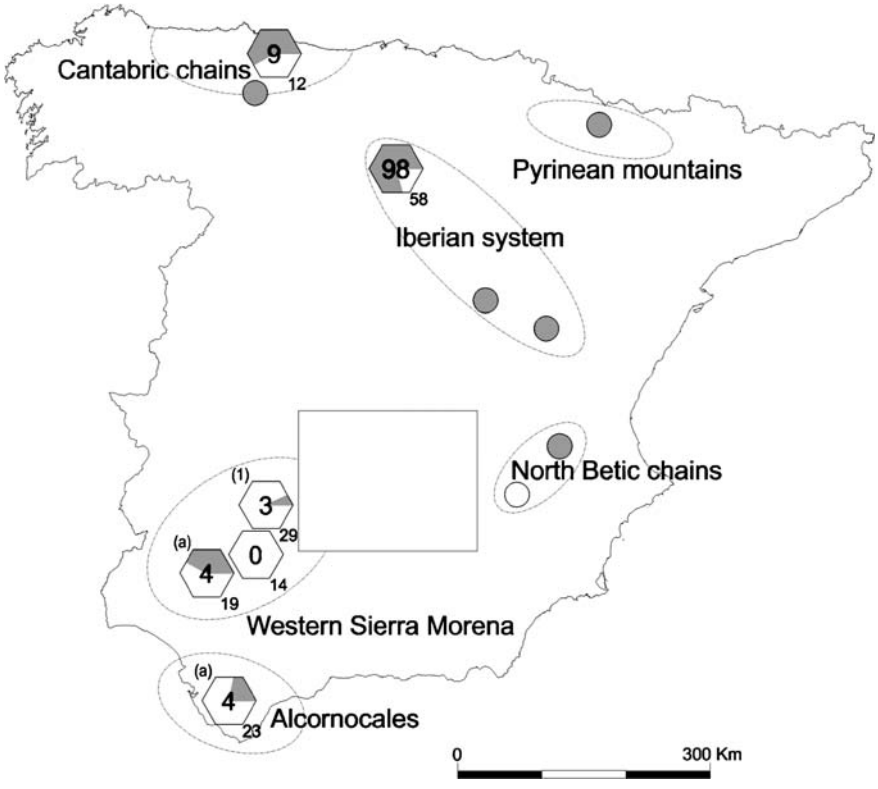

Fig. 1 Map of Spain showing the sampling sites, sample size (on the *right bottom corner* of the hexagon) and *E. cervi* L1 prevalence (percentage of *grey colour* in relation to the whole hexagon represents numerical values) and mean intensity (figure inside the hexagon) from the study populations. *Dots* represent sampling sites where less then ten samples were obtained (*grey colour* when *E. cervi* L1 was found). Sampling sites are the number in *brackets* in the *upper left corners* (see Table 1). *a* Sampling sites where anthelminthic treatment was applied. The *rectangle* represents the core area from South Central Spain where risk factor analysis was performed (see Fig. 2)

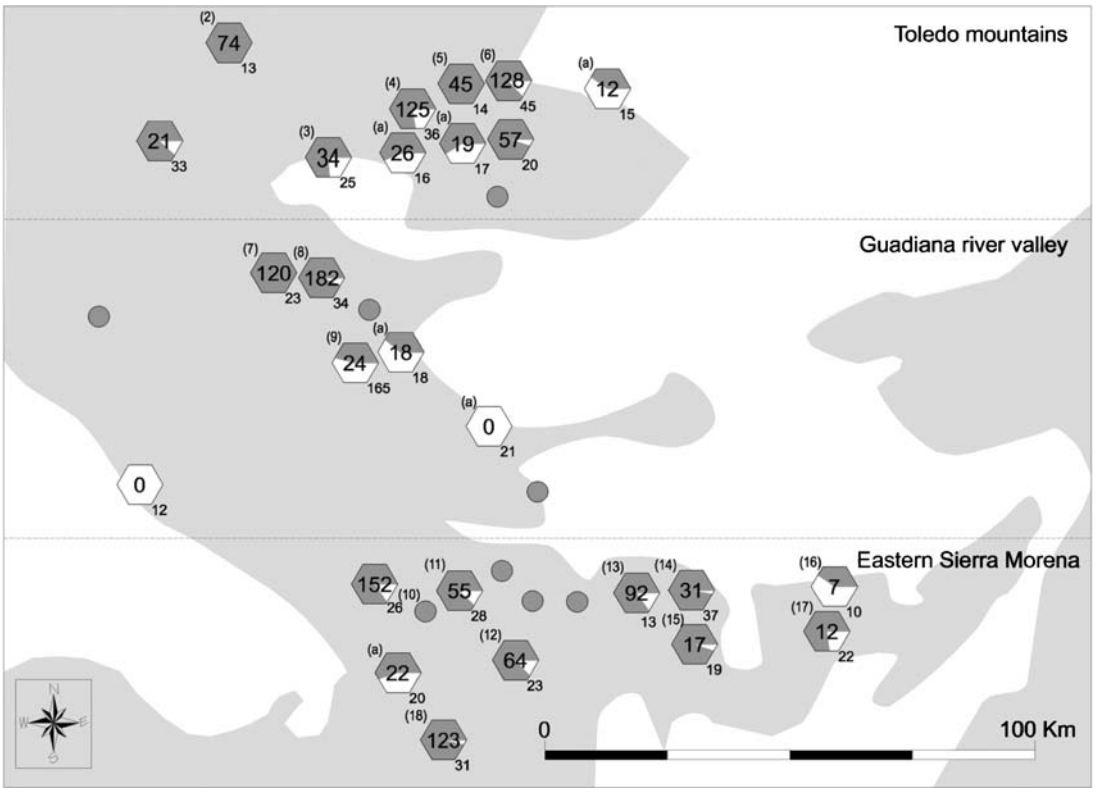

Fig. 2 Map of the core area from South Central Spain showing the sampling sites, sample size (on the *right bottom corner* of the hexagon) and *E. cervi* L1 prevalence (percentage of *grey colour* in relation to the whole hexagon represents numerical values) and mean intensity (figure inside the hexagon) from the study populations. *Dots* represent sampling sites where less then ten samples were obtained (*grey colour* when *E. cervi* L1 was found). Sampling sites are the number in *brackets* in the *upper left corners* (see Table 1) *a* Sampling sites where anthelminthic treatment was applied. Forests and scrublands are represented in *grey*

tailed larvae. The absence of parasitic nodules characteristic of pulmonary protostrongylids at necropsy suggests that no adult worms (except by *Dictyocaulus* spp, which inhabits bronchi and bronchioles) had infected the lungs of the deer examined. In any case, both reindeer and European moose (host to *E. rangiferi* and *E. alces*, respectively) are

Table 1 Overall listing of variables taken into account in the study at the estate level

A. Estate-related features and management practices
General: geographic location, area (hectare), boundaries limiting with other big hunting estates (%), introduction or purchasing of animals (categorical binomial), captures (categorical binomial), predator control (categorical binomial), agricultural lands for hunting (hectare and % related to the estate), fencing status (open/fenced), years since fencing, boundary fenced (%)
Supplemental feeding: supplemental feeding provided for red deer (categorical binomial), feeding method (categorical binomial: spreading or provided in feeders), red deer feeding site density, number of red deer per feeding site (deer per 100 ha/red deer feeding sites density)
Watering: number of red deer watering sites, red deer watering site density, number of red deer per watering site* (deer per 100 ha /red deer watering sites density)
Host demography: red deer kilometric abundance index (KAI) (deer/km), deer density (deer per hectare based on correction from KAI according to Jarvinen and Vaisanen 1975)
Other wildlife: kilometric abundance indices: Mouflon (*Ovis gmelini*, fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), Iberian hare (*Lepus granatensis*), wild rabbit (*Oryctolagus cuniculus*), fox (*Vulpes vulpes*), carnivores other than fox
Dropping or pellet abundance frequency indices: Iberian hare, wild rabbit, carnivore
Livestock: livestock presence (categorical binomial), density livestock (animals per hectare), historic livestock presence (years since), livestock sharing pastures, feeders or watering site with hunting (categorical binomial)
B. Estate-related general environmental conditions (mean values for each estate)
Habitat availability (% and presence/absence) and land cover and habitat structure (each 200 m): Mediterranean scrublands, Dehesa (savannah-like habitat), hardwood forest (*Quercus* spp.), pine plantations, pastures, riparian habitat, agricultural areas. Scrub/forest/grass/soil covers (%), forest and scrub diversity/10 m, number of *Quercus* trees/10 m around, soil compactness (cat.: 1–5)

present in Mediterranean Europe, and the morphology and size of the larvae found in this study, along with the high host specificity of the Elaphostrongylinae, suggest that deer populations studied are parasitized by *E. cervi*. Sex was recorded and age was determined by the tooth eruption pattern and incisor 1 sectioning (n=448; Klevezal and Kleinenberg 1967).

Estimating red deer densities, habitat variables and questionnaires

We visited 18 of the sampling sites in September 2002, immediately before the hunting season, in order to obtain field estimates of red deer relative abundances and habitat variables (Figs. 1 and 2). Red deer relative abundances (kilometric abundance index) were obtained by night-spotting along transect lines and employed as red deer abundance estimates (Whipple et al. 1994). At least one experienced observer participated in each transect.

Habitat and management variables considered in the study were chosen on the basis of their epidemiological plausibility and the local big game characteristics (Table 1). Environmental conditions for the 18 study areas were recorded every 200 m across our lineal transects (n=20 points per estate) to obtain mean values of habitat land uses and habitat structure of each estate. Through a personal interview with gamekeepers, we characterized management practices in each keeping area. We also obtained data on general estate-related features, watering sites (artificial water-hole or natural source), numbers and locations, feeding practices, anthelminthic treatment and presence of other big game species (Table 1). All the variables related to the year 2002. A listing of the variables included in the first step of the risk factor analysis is displayed in Table 1. Summary statistics of the most relevant management practices, land cover and habitat structure characteristics of the estates included in the risk factor study are given in Table 3.

Statistical analysis

The terms prevalence and intensity are used as in Margolis et al. (1982). Quantitative analysis of risk factors for *E. cervi* L1 prevalence and intensity were done using a two-stage analysis to create generalized linear mixed models (GLMMs). First, we correlated at the estate level (n=18) the pairwise association of the hypothesized risk factors and *E. cervi* L1 (%) prevalence and intensity (mean $\log_{10}$+1 *E. cervi* L1 count), respectively, by means of Spearman correlations as a screening step. Calves were excluded from this analysis to obtain comparable data (Table 2). Factors that were associated significantly with the outcome at P=0.01 (established by the Bonferroni adjustment test for multiple comparisons) were considered for further analysis (Table 1). For categorical variables, Kruskall–Wallis analysis was used for simple assessment of associations. We checked for collinearity in the associations between factors that passed the initial screening using chi-square and Spearman's correlation for categorical and continuous variables, respectively. We only included in the final models the most significant variable within each set of collinear factors. These outcome variables were offered into GLMMs.

We constructed GLMMs with intensity and prevalence as response variables (Wilson and Grenfell 1997) that excluded calf age (n=448 after excluding 72 calves and 60 adult individual with unknown age). We designed a GLMM with the individual *E. cervi* L1 intensity (n=320) as a response variable to test the effect of sex (as categorical variable), age (years old, as continuous variable) and sampling month (from October to March, as a categorical variable). The estate (n=18) and sampling year (from 2000

Table 2 *E. cervi* L1 infection rates in estates selected for quantitative analysis of risk factors for prevalence and intensity, and 95% confidence limits

Estate	Number	Prevalence±1.96 SE (95% CI)	Number	Mean intensity±1.96 SE (95% CI)[a]
1	29	6.89±4.79	2	2.76±0.98 (3.32)
2	13	100.00±0.00	13	74.10±74.58 (519.60)
3	25	76.00±170.72	19	34.35±20.59 (178.42)
4	36	77.78±13.77	28	125.56±82.83 (1,055.70)
5	14	92.85±14.00	13	45.47±19.95 (109.17)
6	45	86.67± 10.04	39	128.03±73.97 (1,357.80)
7	23	100.00±0.00	23	120.19±120.76 (1,442.00)
8	34	94.12±8.03	32	181.93±138.11 (2,186.60)
9	165	46.06±76.28	76	23.82±10.38 (350.10)
10	9	77.78± 28.81	7	65.38±97.84 (364.00)
11	32	87.50±11.64	28	55.43?±28.64 (286.70)
12	23	87.00±14.07	20	64.47±27.31 (260.20)
13	13	84.62±20.41	11	92.55±89.52 (495.00)
14	37	97.30±5.30	36	30.96±12.88 (190.43)
15	19	94.74±10.32	18	17.47±7.68 (65.10)
16	10	40.00±32.01	4	6.66±8.72 (19.94)
17	22	77.30±17.92	17	12.03±5.34 (38.23)
18	31	96.77±6.32	30	123.15±53.46 (831.14)

Sampling sites are represented in Figs. 1 and 2

[a]Maximum individual value in the respective estate

Table 3 General statistics for the most relevant land uses, habitat data and management practices at the estate level (n=18)

Variable	Mean	SE	Range
Estate area (Ha)	5,379.71	4,717.02	800–19,328
Estate boundary fenced (%)	86.38	32.89	0–100
Time complete fenced (years since)	18.79	11.10	0–43
Cultivated dehesas or pastures for hunting (estate %)	6.46	9.09	0–29.57
Number of watering sites (number per 100 ha)	0.43	0.26	0.08–0.88
Number of red deer per watering site	117.19	141.12	0–600.00
Red deer abundance index (KAI)	9.70	5.55	2.89–20.64
Red deer abundance (deer per 100 ha)	25.21	18.78	3.41–74.42
Land uses (%)			
Quercus spp forest	23.03	23.01	4.5–63.63
Dehesa	19.41	19.44	056.52
Pine plantation	22.46	26.83	071.42
Pastures	0.40	1.68	07.14
Scrubland	25.49	21.58	065.00
Habitat structure (% in 25 m radio)			
Scrub cover	31.61	9.97	16.77–51.13
Forest cover	23.93	11.66	7.78–50.24
Grass cover	29.36	13.51	7.62–50.65
Soil cover	32.40	10.04	16.79–51.67

to 2004) were considered as random factors. We considered a Poisson error distribution and a logarithmic link function (Wilson and Grenfell 1997). For the presence of *E. cervi* L1 (n=448), we designed a similar model where the outcome variable was defined as the individual's status with respect to *E. cervi* L1 infection (as binomial variable; 0 absence, 1 presence). The number of red deer per watering-hole in the estate was also included. We considered a binomial error distribution and a logit link function. The resulting saturated up to two interactions models were reduced using a backward-selection approach until all variables remaining in the models were significant at P<0.05.

Results

The geographic distribution of the faecal prevalence and mean intensity of *E. cervi* L1 (excluding calves) in red deer populations across the study area are shown in Figs. 1 and 2. Mean prevalence and mean intensity from estates across South Central Spain with at least 10 sampled animals were lower at those where anthelminthic treatments were used (mean prevalence=39.10±13.80%, mean intensity=13.17±6.63, n=8) than at those without treatment (mean prevalence=73.65±13.74%, mean intensity=62.26±22.89, n=22, $Z_{M-W}=-2.747$, P<0.01 and $Z_{M-W}=-2.99$, P<0.05 for prevalence and intensity, respectively). For the study sites

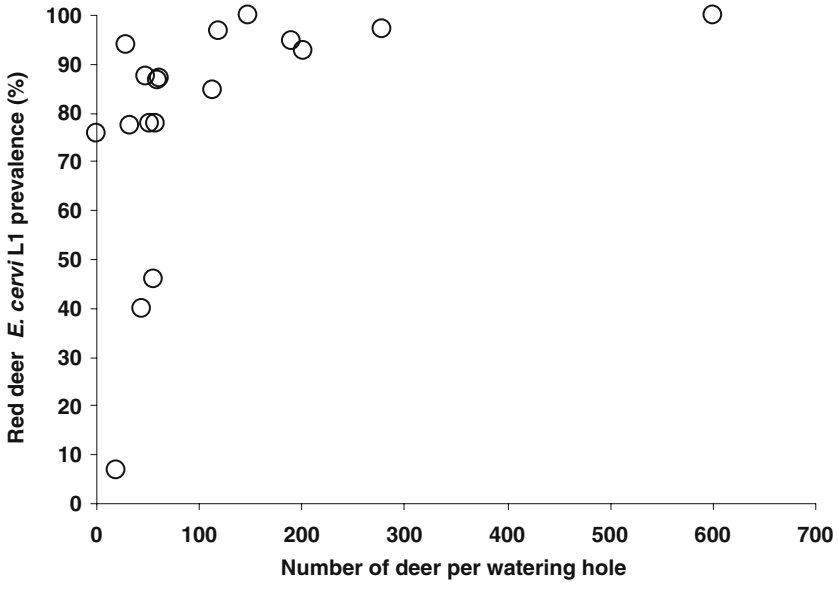

Fig. 3 Relationship between the number of red deer per watering-hole and *E. cervi* faecal L1 prevalence at estate level (n=18)

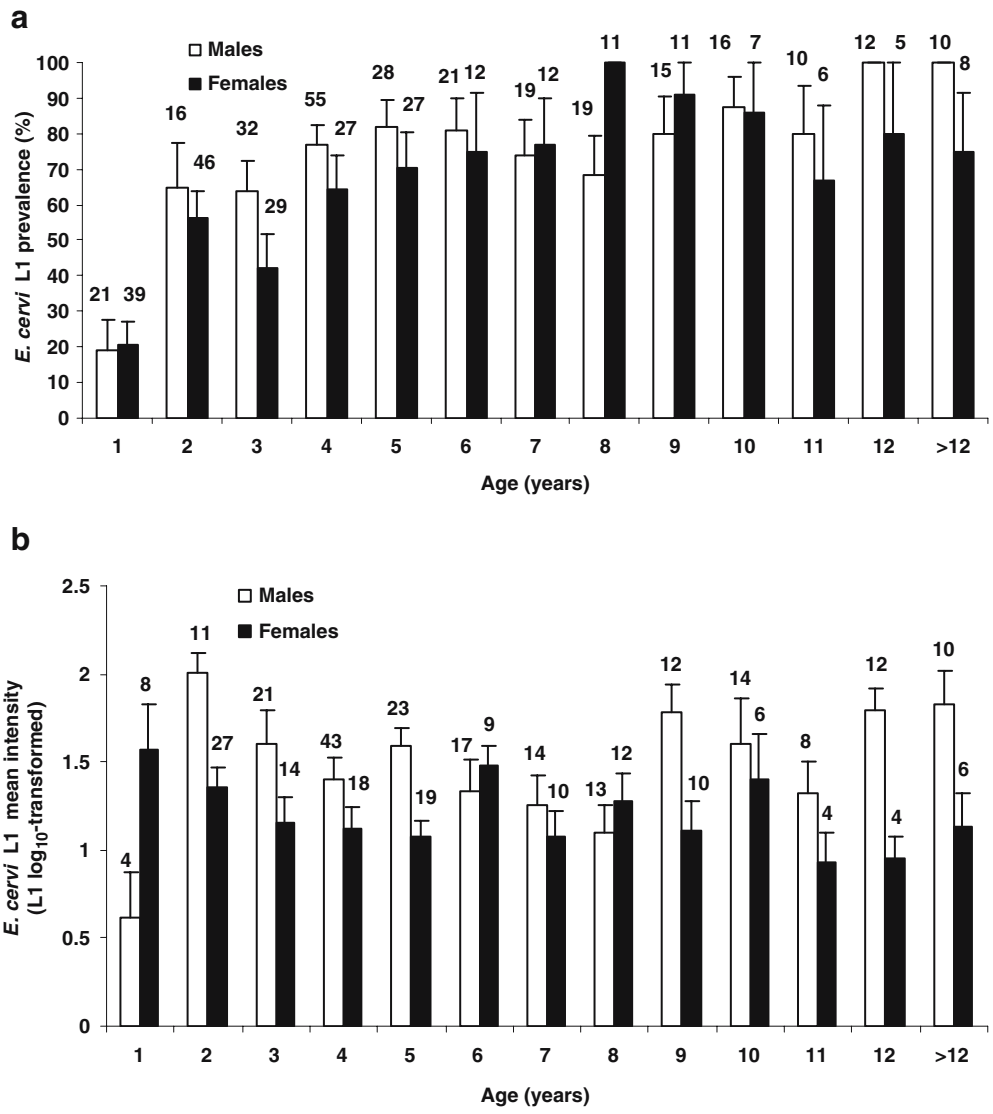

Fig. 4 Sex by age prevalence (**a**) and intensity (**b**) patterns of *E. cervi* faecal L1 in the Iberian red deer from the risk analysis study area. *Vertical lines* indicate 95% confidence limits for prevalence and SE for intensity. Sample size is indicated at the *top of the bars*

used in risk analysis, estate mean prevalence was 79.07± 5.78% (ranging from 6.89% to 100%, n=18) and estate mean intensity was 66.90±12.14 (ranging from 2.76 to 181.93, Table 2). At the individual, mean prevalence was 71.42± 2.14% (n=448) and mean intensity (n=320) was 74.50± 10.35.

Table 4 Results from generalized linear mixed models for *E. cervi* L1 infection rates

Variable	*df*	*F*	*p* value	Estimates
E. cervi L1 presence				
Age	1, 445	21.53	<0.0001	0.22
Deer per water-hole	1, 22.8	2.42	0.05	0.01
Scaled deviance=385.73; scaled Pearson χ^2=434.15[a]				
E. cervi L1 intensity				
Month	5, 130	5.29	<0.0001	−0.17/1.35[b]
Age × sex	2, 311	3.20	<0.01	−0.09/0.00[b]
Scaled deviance=222.59, scaled Pearson χ^2=295.15[a]				

E. cervi L1 presence was modelled using logistic regression (n=448), while intensity was modelled assuming Poisson error distribution and a log link function (n=320)

[a]Fitting statistics and degree of overdispersion of the models

[b]Range of the parameter estimates for categorical variables and their interactions

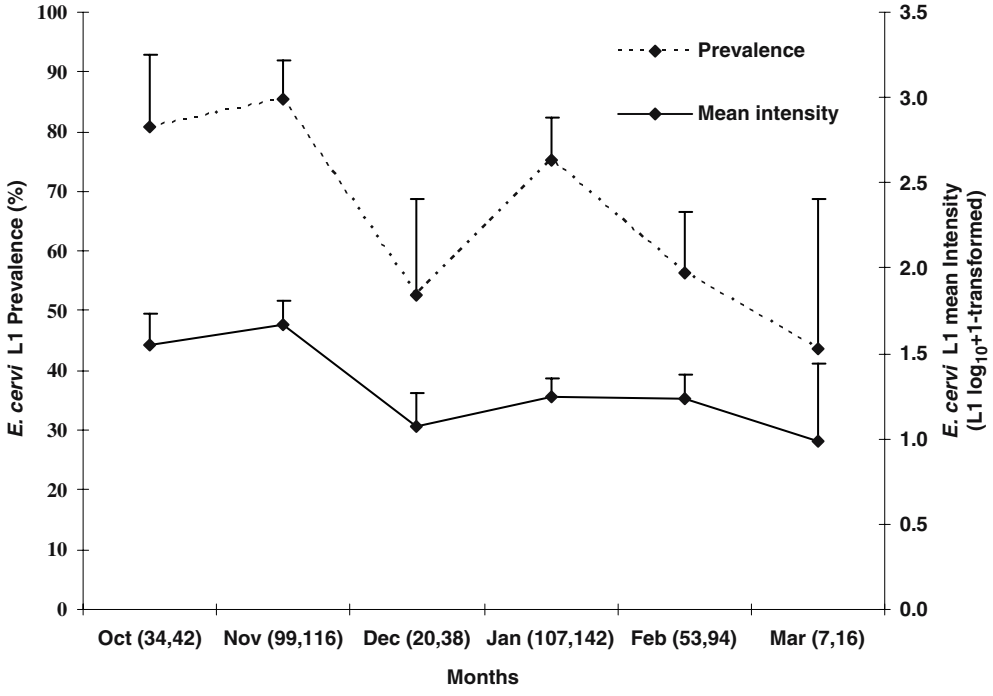

Fig. 5 Monthly variation of mean prevalence and mean intensity of *E. cervi* L1 outputs during the study period. *Vertical lines* indicate SE with 95% confidence limits for prevalence and for intensity, respectively. The sampling sizes for intensity and prevalence, respectively, are shown by the month

Of the variables taken into account in the risk factor analysis, the number of red deer per watering site associated to *E. cervi* prevalence L1 (r_S=0.53, P=0.01, Table 3; Fig. 3). Prevalence was significantly lower for calves compared to elder individuals (χ^2 test, P<0.05; n=60 and n=516 for calves and elder individuals, respectively, Fig. 4). Regarding the animals included in the risk factor analysis, GLMM results for prevalence and intensity of *E. cervi* L1 are shown in Table 4. There was a clear and statistically significant positive age-prevalence pattern (Fig. 3), peaking approximately in 6-year-old animals and then maintaining high rates. The number of red deer per water-hole was significantly related to the probability to excrete *E. cervi* L1.

As regards intensity of *E. cervi* L1 excretion, sex and age interacted significantly. There was a trend towards males having higher intensities than females, but for males more than 2 years old, there was no evidence of a clear age–intensity pattern (estimated slope=0.005, SE=0.02); for females, a slightly decreasing age–intensity profile was identified (estimated slope=−0.1, SE=0.04) (see Fig. 4 and Table 4). The intensity of *E. cervi* L1 counts were significantly related to sampling month, with mean values peaking at October–November (Fig. 5).

Discussion

We found that *E. cervi* is widely distributed among Iberian red deer populations. The parasite occurs in all the sampled areas of Spain; thus, our study expands the known geographic distribution in Southwestern Europe. Finding of infected red deer in Cádiz province establishes the southern limit of *E. cervi* in the Mediterranean Europe. The prevalence figures are similar to those of natural wild red deer populations passing larvae elsewhere in enzootic situations across the geographical range of wild red deer. For example, Demiaszkiewicz (1985) reported that 64 to 91% of red deer were passing larvae upon examination of various hunting grounds in Poland, and English et al. (1985) found 83% in Scotland. As regards the risk factor study area, a wide range of population infection rates were found, even in closely located deer populations. For example, prevalence ranges from 40 to 100% in populations located in the Toledo Mountains area (Fig. 2).

Of factors related to the variability in *E. cervi* L1 excretion across individuals and populations, the main finding in this paper relates to the relationship between prevalence and the level of aggregation of animals at watering-holes. Epidemiological models and comparative data suggest in general a positive relationship between host population density and abundance of macroparasites (Arneberg 2001). Our study suggests that local host aggregation rather than large-scale host density is an important factor in *E. cervi* L1 transmission in our study area. Mediterranean environments are characterized by a drought period in summer, and this may critically affect the survival and spatial availability of larvae in the environment. In addition, summer weather forces gastropods to aestivate or concentrate in humid areas, usually the scarce water-holes that remain along riverbeds. Both the free-living first-stage larvae of meningeal worms and terrestrial gastropods require moisture for movement and survival. Therefore, water-holes are likely to concentrate the spatial and temporal odds of contact between infective *E. cervi* L1 larvae, gastropods and red deer. Due to this apparent dependence of the transmission dynamics of our studied parasite–host system on wet habitats, some parallelism may occur with other major water-borne helminth infections,

such as fasciolosis (Njau et al. 1992). In these studies, prevalence and intensity of infection and the dynamics of transmission vary according to the availability of hosts and to local environmental conditions (Cringoli and Rinaldi, 2003).

An increasing age-prevalence pattern of *E. cervi* L1 faecal outputs was found. Older animals have a higher probability of having acquired infection than younger ones, and *E. cervi* is long-lived in its final host (Watson 1984). Thus faecal presence of *E. cervi* L1 is likely to reflect the way in which deer acquire the parasite across age. Prevalence increased most from calves to yearlings (Fig. 4), with peak prevalence reached in approximately 6-year-olds and maintained thereafter. Similar to our results, prevalence of infection was higher in adult deer than in calves on farms in New Zealand (Mason and Mcalumm 1976).

There was a trend towards males having higher intensities of *E. cervi* L1 than females, but no sex differences in prevalence. Whereas there was no evidence of a clear age–intensity pattern for males more than 2 years old, for females, a slightly decreasing age–intensity profile was identified (Fig. 4). In vertebrate species, it is often found that males tend to harbor more parasites than females (e.g. Alexander and Stimson 1988; Poulin 1996). In our case, the pattern could also reflect a higher female fecundity in male hosts than in females (Stear et al. 1997). This pattern may be due to ecological differences between sexes (Forbes 1993) or to differences in exposure to parasites because of their different feeding habits or habitat use (Wilson et al. 2001). Nevertheless, we could not test if exposure to this is affected by sex in our study area.

The majority of macroparasite systems show age–intensity curves with an initial increase after the age at which an animal becomes susceptible to infection (Wilson et al. 2001). As a result of accumulated experience to parasite antigens, the level of parasite infections may decline due to a protective immune response (Lloyd 1995). Prestwood and Nettles (1977) found in experimental infections of white-tailed deer signs of acquired immunity to reinfection with *Paralephastrongylus andersoni*, which was also suggested for the *Paralephastrongylus tenuis*/white-tailed deer system (Slomke et al. 1995), the reindeer/*E. rangiferi* system (Gaudernack et al. 1984) and the caribou/*E. rangiferi* system (Ball et al. 2001). *P. tenuis* is a long-lived parasite, and most white-tailed deer become infected in their first or second summer of life in Minnesota (Slomke et al. 1995). Interestingly, older male deer (more than 10 years old) showed higher intensities of *E. cervi* L1 excretion than females. The performance of animals may decline with age, but effects of senescence, however, may differ between the sexes because of differences in physiology and behaviour (Saino et al. 2003). Senescence occurs in general earlier in male deer than in females as result of the different reproductive investment (Carranza et al. 2004). We therefore suggest that higher L1 counts in old male deer may be due to senescence affecting immune function. In conclusion, sex by age differences in larvae excretion were evidenced despite the fact that sampling size variation across age classes and months probably affected the precision estimates for larvae excretion rates when comparing sex and ages.

The intensity of *E. cervi* L1 counts was significantly associated with sampling month, with peak values peaking during the October–November period (Fig. 5). The seasonal pattern of faecal excretion of *E. cervi* L1 in Iberian red deer has been monitored previously in South Central Spain (Vicente et al. 2004b). The lowest mean intensity of faecal L1 were found in summer, whereas no seasonal variation was found for prevalence across the year. Consistent with this, we found temporal variation only in the intensity of excretion and not in the prevalence of infection. The seasonal rhythm of *E. cervi* L1 discharge resulted from parasite adaptation to the seasonal Mediterranean climate and habitat constraints to improve the chance of parasite transmission, and positively associated with early coming rainfall (the next month) rather than with rainfall of the same month (Vicente et al. 2004b). Thus our results, with a peak of *E. cervi* L1 intensity of excretion in October–November, are consistent with the autumnal peak of rainfall expected for Mediterranean climate. In addition to climatic factors, individual factors could affect the temporal pattern of *E. cervi* L1 excretion (Gaudernack et al. 1984). Sampling size variation could probably influence results and statistical calculations as regards temporal variation in the intensity and in the prevalence of excretion. In particular, low December values could be biased by insufficient sampling during this month since this decrease in larvae production was not detected when the seasonal pattern of faecal excretion of *E. cervi* L1 in Iberian red deer was previously monitored in South Central Spain (Vicente et al. 2004b).

E. cervi, a sub-lethal parasite of red deer, was found to be widespread across Spanish populations. This points out to the fact that it may constitute a good model to evaluate host–parasite relationships under particular types of management. In particular, water-holes at Mediterranean habitats seem to have an important epidemiological role, which could extend to other infectious agents. In the future, more research on the effect of pathogens on this host species and transmission determinants should be carried out to contribute to the understanding of wildlife diseases in the field.

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