POPULATION ECOLOGY

The importance of parasite life history and host density in predicting the impact of infections in red deer

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Abstract We studied a macroparasite (*Elaphostrongylus cervi*, Nematoda) and a microparasite infection (tuberculosis, TB) in red deer (*Cervus elaphus*) across different populations where managers manipulated host condition, density and aggregation by providing supplemental food. We aimed to test whether and, if so, how persistence and transmission of both parasites differentially varied as host body condition and population density varied. We took account of sex, as red deer life history greatly concerns sex-related traits. Changes in host factors had different consequences for the spread of each parasite type. Individual presence of tuberculosis was positively associated with host density, whereas *E. cervi* abundance negatively related to host density and enhanced body condition. There was lack of body condition density dependence; and body condition was mainly dependent on the amount of supplemental food provided, but also on habitat quality descriptors. Overall, our results suggest that body condition was improved at the cost of increased host contact rates, which implied an ecological trade-off between acquiring resources to cope with *E. cervi*, a macroparasite, and concurrent exposure to mycobacteria. By the simultaneous study of both infections, this research suggests that the effects of changes in host number and population structure on disease spread and persistence need to take into account variation in life histories of the parasites. These findings also raise concern about the ecological consequences of diseases and management of wildlife on host life history.

Introduction

Host individual capacity to deal with parasites is an important fitness-associated trait, which, together with host population density, may account for the variation in parasite transmission and persistence in wildlife populations (Swinton et al. 2001; Wilson et al. 2001). Host defence against parasites requires metabolic resources, which are expected to be highly dependent upon external resource availability. Therefore, parasite resistance of individuals may vary as the body condition varies. The structures of host populations are non-homogeneous in certain properties (Anderson and May 1984; Coulson et al. 2004): the rate of mixing is variable and is positively related to the number of infectious contacts between individuals (Altizer et al. 2003). Nevertheless, hosts are exposed to a diverse array of parasites, and the simultaneous study of infections by pathogens with different life histories should provide insight into the diversity of relationships between parasites, host individual traits and density.

Multiple infections are commonly found in natural populations, and often involve pathogens of different life-history strategies (Crawley 1992). The host–pathogen interactions across life history have molded the virulence

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and specificity of pathogens as well as the defences and life strategies of the hosts (e.g. Pfennig 2001). There are some major factors that distinguish some parasitic agents from others (Dobson 1982). In general, much of the microparasite (viruses, bacteria, protozoa) infection pattern is determined by their ability to multiply directly within the host and a mass-action mode of transmission (Nokes 1992). According to the "law of mass-action", the probability of transfer following contact may depend upon the contact rates and contagiousness of the parasite (the ability of the parasite to establish within a new host, and the susceptibility of the host to the infection). The study of these infections commonly takes the form of compartmentalizing the host population according to infection status. By way of contrast, for infections by macroparasites (helminths and arthropods) the unit of study is the parasite, because they do not reproduce in the host, are longer-lived and less numerous. A number of carefully controlled studies of the impact on their hosts suggest that host mortality rates increase with parasite burden, or that the latter causes significant reductions in the fecundity of infected hosts (Albon et al. 2002; Stien et al. 2002).

Even though the nature of the single host parasite system has been determined in a number of cases, few empirical studies analyse simultaneously the ecology of more-than-one type parasite–host systems (Caron et al. 2003). In this research, we studied two pathogens with contrasted life histories causing infection in red deer, a highly sex-dimorphic species in which sexes strongly differ in life history and resource allocation priorities (Clutton-Brock et al. 1982, Johns et al. 1984, Landete-Castillejos et al. 2004). The pattern of infection by a macroparasite (*Elaphostrongylus cervi*, Nematoda) was studied in parallel to pathogenic mycobacterial infection causing tuberculosis (TB) as a function of population density. We selected deer from populations where host condition and density were manipulated by managers by varying availability of resources (i) to test whether and, if so, how persistence and transmission of both parasites differentially varied as individual body condition and population density of hosts varied. As red deer life history greatly concerns sex-related traits, (ii) we studied how the observed patterns differed across sexes.

Materials and methods

Host–parasite systems

We selected a multi host–parasite system composed of a macroparasite (*E. cervi*) and a microparasite (mycobacteria causing TB), both endemically persisting in red deer populations from South Central Spain (SCS). The extrapulmonary

lungworms *E. cervi* (subfamily Elaphostrongylinae, Cameron 1931) are nematode parasites of the red deer (*Cervus elaphus*) with a wide natural distribution across their hosts (Lankester 2001; Vicente and Gortazar 2001; Vicente et al. 2006a). *E. cervi* intermediate hosts are gastropods containing infective third-stage larvae (Handeland et al. 2000). Extrapulmonary lungworms of cervids have a life span comparable to those of their hosts, and an immunologically determined threshold number of adult parasites remain almost unchanged across age, maintaining a close relationship with host immune defence, resulting in excretion of *E. cervi* larvae 1 (L1) (Gaudernack et al. 1984; Slomke et al. 1995).

Mycobacterium tuberculosis complex infection causes tuberculosis in a wide range of susceptible species (De Lisle et al. 2001). After infection, a chronic wasting disease develops with low induced acquired immunity and low recovery rates. Wildlife populations, usually associated with high abundances, have been found to play a role as maintenance hosts and disease reservoirs (reviewed by O'Reilly and Daborn 1995; De Lisle et al. 2001). Red deer are known to be highly susceptible to TB infection (De Lisle et al. 2001). Recent studies have demonstrated the spread of TB in Spain is linked to situations where wild ungulate species exist at high densities (Gortazar et al. 2005; Vicente et al. 2006b, but see Palmer et al. 2004 as regards *Odocoileus virginianus* in USA).

Host sampling

Autochthonous wild ungulates, specially the red deer, have expanded their range and populations in Spain in the last decades (Gortazar et al. 2000). Traditional big game hunting areas extend mainly through Mediterranean habitats from SCS, where red deer abundances have been increasingly managed. A variety of estates may be found across this area, and they can be classified as semi-free ranging (large enclosures at relatively low densities) and free-ranging cervids (neither enclosed nor intensively managed). To control for ecological variations of the habitat, the area we selected was the province of Ciudad Real and boundaries (Fig. 1; 37°13′48″N–39°31′43″N; 06°34′06″W–2°25′54″W). This area includes Montes de Toledo, the Sierra Morena Mountains and the Guadiana river valley. The climate is Mediterranean. Annual rainfall presents considerable variation (300–700 mm). The wet season typically starts in September–October and concentrates most of the yearly rains. The habitat is Mediterranean, and is characterized by *Quercus* forest (mainly *Quercus ilex*) and scrublands with scattered pastures and small crops. Woodlands across SCS are a continuous distribution of more or less independently managed hunting estates (private or public, including natural preserves).

Table 1 Values (± SE) at estate level for TBL status (estate prevalence, %), *E. cervi* L1 infection rates (mean estate intensity as *E. cervi* L1 count), body condition (Kidney fat index, referred as KFI, %), area (ha), habitat (proportion of pasture, %, and number of *Quercus* spp trees per ha) and distance sampling estimates (no. of counted individuals, no. of counted group, density of individuals and density of groups per ha) across 14 populations of red deer from South Central Spain. Sampling estates correspond with Fig. 1 and sampling size is indicated (*n*)

Estate (*n*)	TB prev (% ± SE)	*E. cervi* (L1/gr ± SE)	KFI (% ± SE)	Area (ha)	Feeding Kg/deer × year	Pastures (%)	*Quercus* (no./ha)	Indiv. (*n*)	Group (*n*)	Deer/ 100 ha	Groups/ 100 ha
1 (25)	4.0 ± 3.9	27.2 ± 8.3	23.5 ± 4.1	19,328	0.0	35.7	15.1	350	95	11.3	3.1
2 (18)	38.9 ± 11.5	13.3 ± 5.2	49.2 ± 10.0	2,600	419.3	5.0	15.3	319	53	90.1	19.2
3 (36)	19.4 ± 6.6	97.6 ± 33.9	34.2 ± 4.2	6,862	0.0	34.6	28.8	295	65	29.6	9.4
4(14)	14.3 ± 9.4	10.8 ± 5.0	35.3 ± 4.6	3,000	78.2	14.3	11.2	172	40	32.8	7.1
5 (32)	6.3 ± 4.3	136.6 ± 45.3	22.8 ± 3.3	1,700	14.8	30.0	21.4	163	58	19.8	8.0
6 (12)	25.0 ± 12.5	4.8 ± 4.4	16.2 ± 0.9	1,100	13.1	41.2	0.6	145	32	55.7	12.4
7(18)	5.6 ± 5.4	133.2 ± 78.6	18.7 ± 2.2	6,118	0.0	5.0	2.0	142	42	17.3	5.5
8 (25)	0.0 ± 0.0	103.3 ± 31.1	14.8 ± 1.4	2,090	0.0	19.0	3.6	82	33	16.9	8.1
9 (137)	19.7 ± 3.4	10.9 ± 2.9	41.9 ± 2.6	860	430.3	56.5	4.9	212	49	43.4	9.6
10 (15)	0.0 ± 0.0	8.3 ± 3.5	23.4 ± 5.6	700	69.8	35.0	12.7	67	32	12.3	7.5
11 (17)	0.0 ± 0.0	37.7 ± 14.8	21.2 ± 1.9	2,200	0.0	18.2	20.6	56	21	18.3	6.7
12 (11)	0.0 ± 0.0	87.3 ± 46.5	46.3 ± 8.8	10,700	0.0	50.0	43.8	199	46	26.5	6.8
13 (12)	0.0 ± 0.0	16.9 ± 3.8	13.8 ± 1.3	1,000	0.0	20.0	8.4	125	54	25.7	10.9
14 (33)	0.0 ± 0.0	30.5 ± 7.2	27.3 ± 3.7	3,100	0.0	5.0	9.2	275	102	32.4	13.7
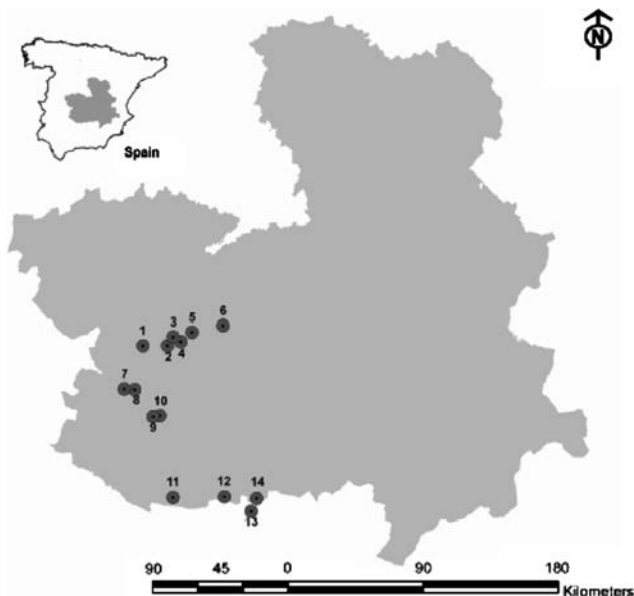
Fig. 1 Map of South Central Spain (Region of Castilla-La Mancha in dark) showing the sampling sites (numbers correspond with Table 1)

For sampling, we deliberately chose 14 sites where *a priori* a range of deer densities would be present (Fig. 1; Table 1). These densities are highly variable, and may reach high values due to game management, probably over the natural carrying capacity in some estates. Information from animals coming from these sites was previously employed to evaluate the epidemiology and risk factors in elaphostrongylosis and TB infections, and the results are presented in separate papers (Vicente and Gortazar 2001; Gortazar et al. 2005, Vicente et al. 2005, 2006a, b).

Samples were collected from carcasses (*n* = 405) recovered during the normal hunting seasons (November–February) from 1999/2000 to 2004/2005. These large beats involve up to 100 hunters and several groups of dogs, covering areas of over 400 hectares. Within the possibilities which hunting results offered, we arbitrarily selected a random age- and sex-stratified subset of animals. Sampling in the winter months also had the advantage of standardising seasonal variation in parasite prevalence or intensity of excretion (Vicente et al. 2005).

Each deer was necropsied in the field. Age was determined from incisor-1 sections as described by Klevezal' and Kleinenberg (1967). We estimated body condition using the kidney fat index (KFI), since the whole body fat levels in cervids correlate with KFI (Finger et al. 1981). Fresh faecal samples were collected from the rectum. Protostrongylid larvae were extracted in less than 24 h from 8 g of faeces using the modified Baermann method (Forrester and Lankester 1997), and expressed as number of larvae per gram of faeces (lpg). L1 were identified on the basis of biometry and morphology (Vicente and Gortazar 2001). TB was diagnosed by detailed macroscopic inspection of lymph nodes, including parotidean, retropharyngeal and submandibular lymph nodes in the head; tracheobronquial,

mediastinic lymph nodes and lungs in the thorax; and mesenteric and hepatic lymphnodes, ileocecal valve, kidneys, liver and spleen in the abdomen (Vicente et al. 2006b). Gross lesions in other locations were also recorded. Lymph nodes were collected, dissected and sectioned serially and examined carefully for gross lesions in the laboratory. Animals were determined to be positive for TB if they had lesions that were consistent with TB infection (Rohonczy et al. 1996). We only analysed animals from areas where TB infection was supported by isolation in Coletsos medium and confirmed by PCR. These results are reported in a separate paper (Gortazar et al. 2005).

Population parameters

We visited the sampling sites in September 2002 in order to obtain field estimates of red deer density by means of night-spotting and distance sampling methods (Burnham et al. 1980; Whipple et al. 1994; Buckland et al. 2004; Distance 5.0 software). Red deer were counted along 2 km long transects in each estate (which represented a mean of 10 transects per estate, a mean length 21.22 ± 6.85 km per estate, and an effort of 5 km/1,000 ha) (Burnham et al. 1980). These transects were to represent vegetation cover units of the respective estates. At least one experienced observer (JV, CG) participated in each transect. The counts began 30 min after dusk at an average speed of 10 km/h. A 100w-spotlight was handled by the co-driver. Low accurate and precise estimates of deer density by distance sampling methods might represent variation in group size caused by differences of habitat cover among sites rather than variation in density per se (see Table 1). We did not find any correlation between mean group size and habitat variables (see below for estimates of the proportion of pastures, scrublands and *Quercus* forests in the estate, and Vicente et al. 2006a), which suggests that our estimates were reliable enough to compare between localities. The Hazard Rate Model with no expansion term was selected based on AIC value (Buckland et al. 2004), goodness of fit (χ^2) and visual comparison of histograms. Data were manually grouped with an initial interval of 35 m followed by 20 m intervals, and were truncated at 255 m to improve model fit by removing outliers. Data were analysed as clusters. Multiple covariate distance sampling was used to examine the effect of habitat type on detectability of animals. This did not improve model fit; however, these data were only available for a proportion of observations. Habitat and management variables considered in the study were chosen on the basis of their ecological value to explain individual condition and habitat effects on the different patterns of infections across deer, according to local big game characteristics and constraints of Mediterranean habitats to red deer biology (a complete description can be see in Vicente et al. 2006a).

Environmental conditions for the 14 study areas were recorded every 200 m across our linear transects (usually $n = 20$ points per estate). These transects were previously designed as stratified to obtain representative mean values of habitat land uses and structure of each estate. Together with Mediterranean scrublands and forests, "dehesas" are the most characteristic elements of the Mediterranean landscape in South Central Spain. They are high productive (but seasonal) savannah-like open habitats composed mainly by pastures and scattered trees (*Quercus* spp, evergreen oak as predominant species). We quantified the availability of pasture habitat across estates as the proportion of the sampling points classified as dehesa and/or pasture (%). This rate is also an index of how predominant the open areas are in relation to Mediterranean scrublands and forests. At each point, we estimated the mean number of *Quercus* spp trees (quantified as the number of *Quercus* spp over 4 m tall in a 5 m radius around sampled points) to characterize availability of Mediterranean productive woodlands across the different estates. Gamekeepers were interviewed in order to characterise food-provisioning practices in each keeping area. We define food supplying as the provisioning of cereal and leguminous material in grain and/or in pellets. Summary statistics of the infection rates, management practices and habitat characteristics of the estates included in the statistical analysis are given in Table 1.

Statistical analyses

Factors that affect infection

This study includes animals older than 2 years. Based on the individual, we conducted analyses for the factors affecting parasite counts (faecal *E. cervi* L1 abundance) and TB status (presence of tuberculosis-like lesions, TBL) by means of generalized linear models (GLMs, Crawley 1993). The first model included individual *E. cervi* L1 counts as a response variable. Sex (as categorical variable, 1 male, 2 female), TB status (as categorical variable, 0 TBL negative, 1 TBL positive) and KFI (as continuous variable, %) were included as explanatory variables. As an index of population density, we considered the distance sampling estimates (Table 1) as a continuous variable. The amount of food provided by managers was quantified as kg supplied per deer and year in each estate (Table 1). Because of the absence of data between 100 and 400 kg per deer and year, the amount of food provided by managers was treated as a two-level factor (low feeding = 0 when <100 kg peer deer and year, and high feeding when >400 kg per deer and year). Habitat variables considered in the analysis included the mean number of *Quercus* spp trees (as continuous variable) and the proportion of dehesa-pastures in the estate (as continuous variable, %). We controlled for the population

and for the sampling year, both as random factors. We considered a Poisson error and a logarithmic link function (Wilson and Grenfell 1997) for *E. cervi* model, and a binomial error and a logistic link function for TB model.

Factors that affect host body condition

We evaluated the relationship between body condition (KFI, as continuous response variable) feeding and host abundance controlled by the effect of sex, TBL status and *E. cervi* L1 counts. We also included both habitat variables as explanatory. This model was fitted with a Poisson error and a logarithmic link function.

The resulting models, saturated up to two interactions, were reduced to their simplest form by eliminating in a backward stepwise manner any explanatory variables or interactions that failed to explain significant variation in the response. The best-fit models were identified using the Akaike Information Criterion (Burnham and Anderson 1992). The level of significance was established at the 5% level. Statistical parameters and phenotypic traits in the text are expressed with $SE_{95\%C.I}$.

Results

TBL prevalence for the total sample was $12.35 \pm 3.17\%$ ($n = 405$). *E. cervi* L1 prevalence was $68.89 \pm 4.51\%$, and mean abundance was 45.52 ± 13.19 lpg, showing overdispersion (variance to mean ratio = 403.01). Mean body condition (KFI) was $31.75 \pm 2.61\%$. The average values for *E. cervi* infection, TB and KFI per estate are shown in Table 1, together with density estimates, feeding management and habitat values. Six out of 14 estates supplemented food.

Factors that affect infection

The results of the specific models are shown in Table 2. Males ($n = 223$) presented higher *E. cervi* L1 excretion rates than females ($n = 182$) (abundance: $68.49 \pm 11.64\%$ and $17.35 \pm 3.63\%$ for males and females respectively; prevalence: $76.68 \pm 5.56\%$ and $59.34 \pm 7.15\%$ for males and females respectively). We found a statistically significant association between parasite abundance and body condition; the higher the body condition, the lower the *E. cervi* L1 counts (Fig. 2a). Individual counts of *E. cervi* L1 statistically correlated with estimates of host density; the higher the density, the lower the excretion values (Fig. 2b). Concerning the model on TB, individual risk of presenting TBL showed an opposite trend to *E. cervi*, and positively associated with estimates of host density (Fig. 2b). No statistical differences in the risk of presenting TBL were found between males ($12.56 \pm 4.35\%$) and females ($12.09 \pm 4.74\%$).

Table 2 Generalized linear models for *E. cervi* L1 abundance and tuberculosis-like lesion presence respectively

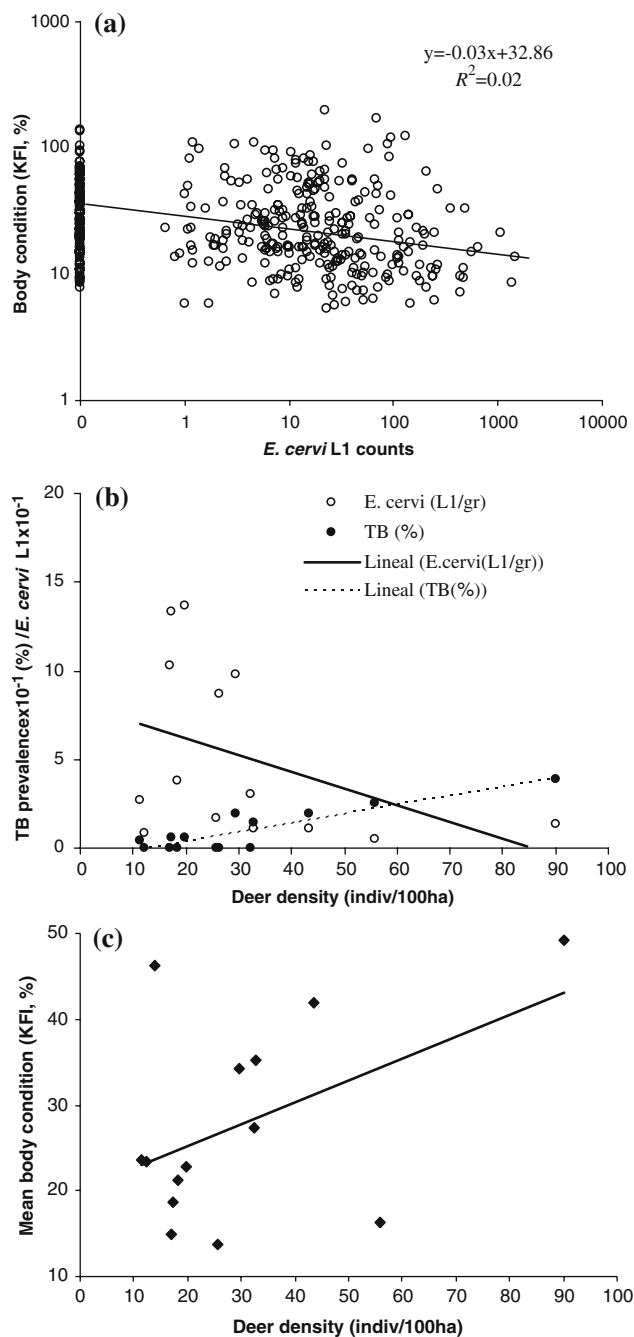| Variables	df	F	P value	Estimate
E. cervi abundance				
Sex	1,397	14.25	<0.01	0.97
TB presence	1,387	1.36	0.24	0.41
Kidney fat index	1,397	5.04	0.02	−0.04
Density	1,36.4	6.00	0.02	−0.05
Pastures	1,14.2	0.26	0.62	0.85
Kidney fat index × pastures	1,394	1.36	0.24	0.03
Kidney fat index × density	1,384	7.21	<0.01	0.00
Tuberculosis presence				
Sex	1,381	0.26	0.61	−0.40
Density	1,7.87	8.21	0.02	0.03
Sex × density	1,397	1.26	0.26	0.02

For *E. cervi* L1 counts, the distribution error function is the Poisson with a logarithmic link function, and for the TB model the distribution error function is the binomial with a logistic link function. Parameter estimates for the levels of fixed factors were calculated considering a reference value of 0 for the level 1 (positive) in the variable "TB presence" and for the level 2 (female) in the variable "sex"

Factors that affect host body condition

The results of the fitted model are shown in Table 3. Females showed higher KFI scores ($42.52 \pm 4.09\%$) than males ($22.96 \pm 2.87\%$). There was a statistical positive relationship between the amounts of supplied food (as categorical) and body condition (Fig. 3a), whereas density was dropped off the model [the relationship between mean body condition and deer density across estates was not significant—see Fig. 2c; the positive correlation between the amount of food supplied and the density ($R_S = 0.51$, $P = 0.06$, $n = 14$)was marginally significant]. It is also noteworthy that deer from estates where supplemental food was provided ($n = 228$ from 6 estates) scored a mean KFI of $36.83 \pm 1.97\%$ (26.41 ± 5.23, $n = 104$, and 45.57 ± 5.14, $n = 124$, for males and females respectively), whereas deer from estates where this practice was absent ($n = 177$ from 8 estates) scored $25.21 \pm 3.01\%$ (19.95 ± 2.78, $n = 119$, and 35.98 ± 6.39, $n = 58$, for males and females respectively). Availability of *Quercus* spp tree cover related statistically and positively to body condition (estimate parameter = 0.14, Table 3). Sex-related differences mediated to determine a more marked negative relationship between body conditions and *E. cervi* counts in males (Fig. 3b).

Discussion

We assessed the relationships between infectious agents with contrasted life histories and deer body condition and

Table 3 Generalized linear models for kidney fat index

Variables	df	F	P value	Estimate
Sex	1,355	22.17	<0.01	−0.33
Supplied food	1,6.07	59.08	<0.01	−0.56
Quercus trees	1,12.5	31.60	<0.01	0.14
Sex × *E. cervi*	2,388	3.23	<0.01	−0.03 (male) 0.19 (female)

The distribution error function is the Poisson with a logarithmic link function. Parameter estimates for the levels of fixed factors were calculated considering a reference value of 0 for the level 2 (female) in the variable "sex" and for the level 1 (low feeding) in the variable "supplied food"

an example to illustrate how pathogens of diverse nature (microparasites and macroparasites) can very differentially interact with a host, since parasite transmission and persistence is a complex interplay between both host and parasite life history and host density. Changes in host factors had different consequences for the transmission and persistence of each parasite species, which means that the interpretation of these ecological relationships needs to take into account variation in the host parasite system's life history.

E. cervi L1 counts and body condition associated negatively, which is what one would expect considering that individuals in prime body condition would invest more (or more efficiently) in antiparasitic defences (Moller et al. 1998; Lochmiller and Deeremberg 2000). On the other hand, *E. cervi* L1 counts and host density associated negatively. Epidemiological models and comparative data predict a positive relationship between host population density and abundance of macroparasites (Arneberg 2001), since parasite transmission rate is a positive function of host population density (i.e. contact rates) and high host density would increase the susceptibility to *E. cervi* infection in natural conditions. One explanation for the negative relationship between host density and parasite infection rates is that improved body condition (or increased resistance against parasites by other means) positively relates to density in our study populations. In Mediterranean habitats from SCS, high deer population densities are maintained by artificially increasing the carrying capacity with supplemental feeding, and then deer density becomes a positive correlate of the intensity of feeding practices (Sanchez-Prieto et al. 2004). Therefore, a disproportionly better condition is expected in animals from more intensively managed populations, and a subsequent improved resistance against parasites (models confirmed that individual body condition was positively related to the estimated amount of food provided per capita). This prediction tested positively for the macroparasite *E. cervi*, and is consistent with previous research in extrapulmonary lungworm infections in cervids, where host immune response modulated the establishment and fecundity of parasites (Gaudernack et al. 1984).

Fig. 2 a Relationships between individual *E. cervi* counts (lpg) and body condition (kidney fat index, %). The axis scales are logarithmic; therefore ticks are not regularly spaced. It should be noted that in this regression scatter plot the partial effects of the other explanatory variables upon the dependent ones are not accounted for. R^2 was 0.04 for the fitted regression for infected deer only. **b** Relationships between deer density (deer/ha) and mean estate abundance of *E. cervi* L1 counts (lpg 10^{-1}) ($R_S = -0.41$, $P = 0.1$) and TBL prevalence (% 10^{-1}) ($R_S = 0.67$, $P = 0.01$) respectively. **c** Relationships between mean estate body condition (%) and deer density (deer/ha) ($R_S = 0.49$, $P = 0.07$)

population density, accounting for the effects of resource variability (food provisioning by managers) on host condition and aggregation. Our particular case could be taken as
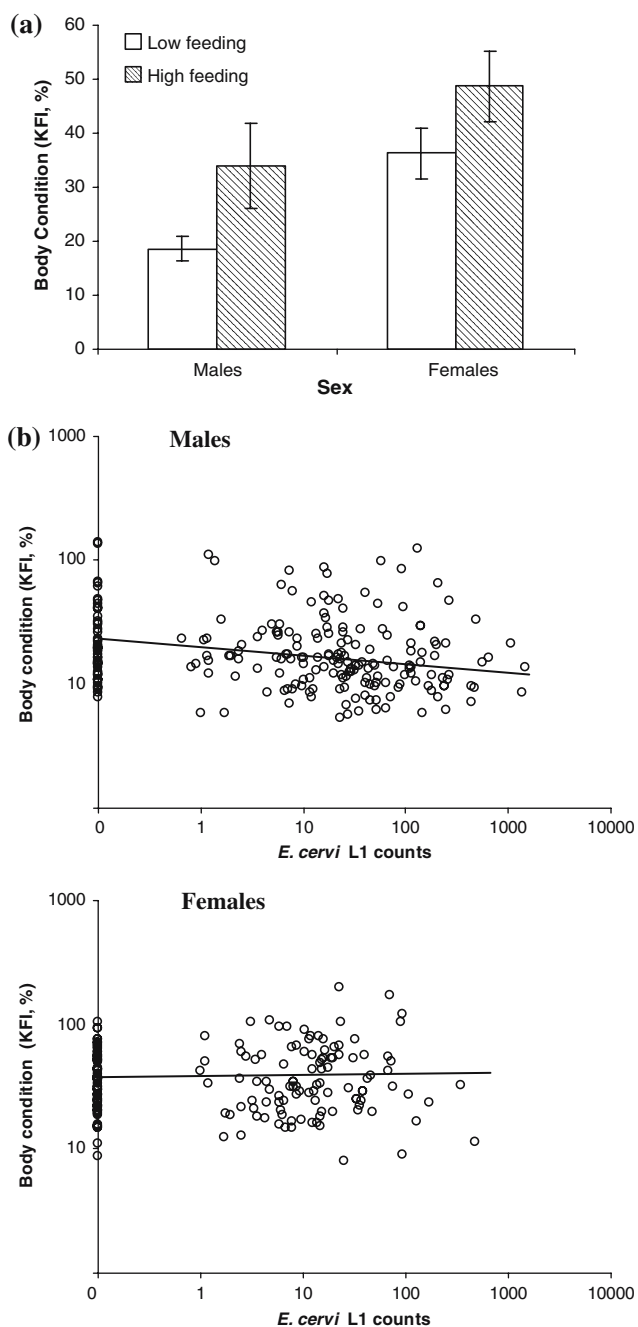
Fig. 3 Relationships between body condition and **a** supplemental food provided, treated as a categorical two-level factor [low feeding = 0 when <100 kg per deer and year, and high feeding when >400 kg per deer and year; expressed as mean ± SE (95% confidence interval)]; and **b** *E. cervi* L1 counts, accounting for sex (%). The axis scales are logarithmic; therefore ticks are not regularly spaced. Sex by *E. cervi* counts remained as significant terms in the fitted model. It should be noted that in this regression scatter plot the partial effects of the other explanatory variables upon the dependent ones are not accounted for

On the other hand, concerning the pattern and factors influencing TB infection, we found a positive relationship between host density and the presence of TB across individuals,

which is what one would expect according to a mass–action system of transmission of directly transmitted microparasites (Swinton et al. 2001). Improved individual body condition did not attenuate risks of testing positive to TBL. Since it is expected that a good body condition by itself does not increase the risk of presence of TB, our findings suggest that risk for TB transmission could be due to population factors somehow associated to improved body condition. Foraging could relate to increased risk of disease transmission when this behaviour is positively associated with direct or indirect host contact rates (Swinton et al. 2001). This additional risk may be a consequence of a common practice in our study populations: artificial provisioning at feeding places (and even of natural feeding behaviour). Artificial provisioning increases the carrying capacity and facilitates host contacts. Contrarily to *E. cervi*, transmission of mycobacteria may disproportionably rely on increased aggregation and/or contact rates at feeders (Palmer et al. 2004) rather than on a more complex and coevolved life cycle (Vicente et al. 2006a, b). Other feeding areas in Mediterranean landscapes, such as pastures or seasonal production of acorn by woodlands (mainly *Quercus* spp) (see below), could aggregate hosts and facilitate TB transmission, but we failed to detect any relationship between TB and habitat-related factors in this research. Mycobacteria cause chronic disease, but induce low immunity and recovery rates (Morris et al. 1994). Interestingly, the spread of TB depended on infectious events associated to artificial feeding, and TB presence was independent of deer body condition. This schematic and simplified explanation of mechanisms does not imply that other determinants known to be involved in host-parasite interactions do not operate. Nevertheless, we identified a life-history interaction with density, and our findings also confer a practical value on the study of different pathogens across managed wildlife populations.

We defined individual deer as natural sampling units of habitats for pathogens in which mixed infections could occur, but there is no general theory about how micro and macro parasites might interact. Parasite interactions within the hosts can either be ecological by competition for space or resources (but we selected two pathogens that work at very different spatial scales) or immunological (the infection by a particular parasite may modulate the immune response against other parasites, Cox 2001). Whilst many microparasites escape immune attack by antigenic variation or sequestration in specialized niches (mycobacteria within host macrophages), many helminths appear to thrive in exposed extracellular locations (Maizels et al. 2004) (although cell immunity could be important for some parasites, such as gastrointestinal nematodes, Huntley et al. 1995; Stear et al 1999). Chronic worm infection could even affect resistance/susceptibility to mycobacterial infections,

by impairing mycobacteria antigen specific cellular responses (Stewart et al. 1999; Elias et al. 2005). We did not find any association between the abundance of the macroparasite and the presence of TB across individuals, which suggests that if any interaction exists, this is not driven by within-host interaction, but could relate to the structure of contacts at population level and particular host–parasite life histories (as discussed above). The animals included in this study were apparently healthy, but terminal cases of tuberculosis (Caron et al. 2003) or other wasting situations (Höfle et al. 2004) probably lead to an exacerbation of concomitant infections. It is worthy of note that if any bias at sampling existed, this would favour double-infected animals, since they may be less active or otherwise peculiar in their behaviour, but this aspect could not be assessed in this study.

Concerning the generalization of our results on *E. cervi* to other macroparasite infections, the response to host densities, contact structure and condition could not vary quantitatively in a similar degree for all parasites found in the community (Wright and Gompper 2005), particularly as the transmission mode is direct for some other helminth species. A positive relationship between host density (and aggregation) and parasitism is expected particularly for directly transmitted parasites. A further major advantage for nematodes of complex life (a parasite that utilizes two or more species of host in sequence cycles) is that they usually reduce the host abundance threshold at which the parasite is able to establish (Dobson et al. 1992). This allows the parasite to exploit hosts that may only visit the parasite's habitat for short periods of time (Vicente et al. 2006a), and would make parasite transmission less density-dependent when compared to parasites with direct life cycles. The mechanisms of host immune response against different helminths to reduce their establishment, survivorship and fecundity are similar in part (e.g. Paterson and Viney 2002; Maizels et al. 2004), which suggests that the described pattern for *E. cervi* could present similarities to other low pathogenic helminths, especially those with indirect life cycles. Concerning TB, there are a wide variety of patterns of behaviour in microparasites. The most typical are short infectious periods and the induction of lasting specific immunity in hosts in many viral and bacterial infections, the community size being critical (e.g. Swinton et al. 1998). Mycobacterial infections present particularities: they generally develop into chronic infections, with long-term persistence in populations and low induced immunity. Mycobacteria are able to induce a period of infectiousness during which direct contact between individuals occurs, which may favour transmission by both direct and indirect contact (Morris et al. 1994; Walther and Ewald 2004), the structure of contacts between hosts being more critical for its transmission and persistence (Delahay et al. 2000).

Males presented higher *E. cervi* L1 counts than females. This finding is consistent with previous studies, where males tended to exhibit higher rates of parasitism and disease and showed reduced immune responses compared with females (e.g. Alexander and Stimson 1989; Zuk 1990; Schalk and Forbes 1997). The immunocompetence handicap hypothesis postulates that males may have higher parasite loads than females due to the immunosuppressive effects of testosterone (Folstad and Karter 1992). Alternatively (or additionally), sex differences in parasitism may be due to differences in the susceptibility to parasites caused by the different roles of males and females in activities related to sexual selection, which may diminish the amount of resources available to immune function during mating in the former (Alexander and Stimson 1989; Zuk 1990). Thus, sexual selection could lead to the observed higher parasite levels in males compared to females via differential resource investment and/or via immunosuppressive effect of androgens. The finding that males presented lower body condition, and a stronger relationship between *E. cervi* L1 counts and body condition that was found for males (Table 3), may relate to resource investment in male–male competition and hierarchy establishment during rut (which took place just before data collection) (Johns et al. 1984) rather than in anti-parasite defence, which could lead to increased excretion of *E. cervi* L1 (Gaudernack et al. 1984). Additionally, there could be a disproportional effect of parasites on male body condition as compared to females (the directions of the effects are not testable in our correlational study).

By the simultaneous comparison of both infections, we showed that the effects that changes in host number and population structure (and probably host interactions) have on disease transmission and persistence need to take into account variation in the parasite life history. Our findings also raise concern about the ecological consequences of diseases and the management of wildlife on host life history.

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