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Epidemiology of cerebrospinal *Elaphostrongylus cervi* infection in red deer in central Spain

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Abstract

Elaphostrongylus cervi produces a subclinical cerebrospinal disease in many wild and domestic ruminants from Europe, North America and New Zealand and has recently been described in Spain. To determine some aspects of its epidemiology, 121 red deer (*Cervus elaphus*) from central Spain were sampled during 2000. The prevalence (7%) and mean worm burden (3.8 worms per brain) were similar to the values previously recorded in other European areas. The infection was only detected in young deer during the winter. The estimation of larval production in the faeces was not a reliable method of diagnosing *E. cervi* infection.

Introduction

Little is known about parasitic infections of red deer in the Iberian Peninsula. In consequence, since 1997 the Servicio de Investigación y Tecnología Agraria de Castilla-La Mancha have sampled more than 400 *Cervus elaphus* from this region (project INIA no. SC97-034) to determine the parasite fauna of this cervid. During the development of this project, nematodes found in the central nervous system were identified as *Elaphostrongylus cervi* (Protostrongylidae: Metastrongyloidea) (Valcárcel & García Romero, 2002).

The genus *Elaphostrongylus* is a parasite of several species of Cervidae. This nematode produces a subclinical cerebrospinal disease (Mickevich, 1958; Bakken *et al.*, 1975) but can cause serious clinical signs (Watson, 1983; Handeland *et al.*, 2000b) and even death (Handeland & Norberg, 1992) and it can also infect domestic small ruminants (Handeland & Slettbakk, 1995; Pusterla *et al.*, 1998a,b; Handeland *et al.*, 2000a). *Elaphostrongylus* is mainly described in wet areas of Europe, North America and New Zealand, which are colder than Spanish conditions.

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E. cervi was recorded for the first time in Spain by Valcárcel & García Romero (2002) so no data is available on elaphostrongylosis. The aim of present study is to describe the prevalence and seasonality of *E. cervi* in a mid-wet area of central Spain.

Material and methods

Sampling sites

Red deer (*Cervus elaphus*) were collected from three provinces of Castilla-La Mancha, Toledo, Ciudad Real and Cuenca, where the density of red deer is high. The deer were divided in two groups depending on the climatic characteristics of the sites (fig. 1). The first site, which includes Toledo and Ciudad Real is a typical Mediterranean tableland with a semiarid climate, an annual rainfall of 355.3 mm, and a mean temperature of 15°C. The second site, which includes two game reserves of Cuenca, is a typical Continental mountain forest with an annual rainfall of 469 mm and a mean temperature of 13°C. One game reserve, the Hosquillo National Park, is a fenced and controlled reserve (910 ha, 12.42 red deer km^{-2}) whereas in the second, the Reserva Serranía de Cuenca (24.814 ha, density of 3.70 deer km^{-2}) which surrounds the Hosquillo National Park, deer are not controlled.

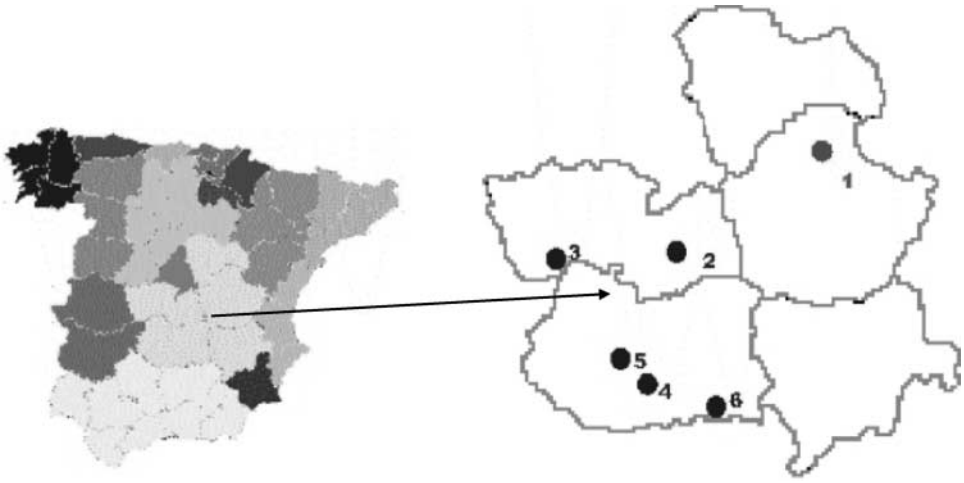

Fig. 1. Sampling sites for red deer from a Continental mountain forest (1, Las Majadas Cuenca n = 86) and a Mediterranean tableand area (2, Los Yebenes (Toledo) n = 3; 3, Sevilleja de la Jara (Toledo n = 4; 4, Abenojar (Ciudad Real) n = 1; 5, Almuradiel (Ciudad Real) n = 17; 6, Piedrabuena (Ciudad Real) n = 10) in central Spain during the periods January to December 2000 (Cuenca) and October to February 1997 to 2000 (Toledo and Ciudad Real).

Deer sampling

Authorization from the local Servicio Regional de Caza was obtained to establish a regular sampling method from January to December 2000 in Cuenca province (n = 86, at least six deer per month with the exception of January and December when only five animals were captured). Deer from Toledo and Ciudad Real were sampled during the hunting seasons (October to February) of 1997–2000, and there were difficulties in examining brains due to the economic value of the trophies. Thus, in these provinces sampling was irregular and the number of heads examined was limited (n = 35).

The examination of heads from Toledo and Ciudad Real took place 4–24 h after death. Deer from Cuenca were sampled immediately after they had been shot by guardians of the game reserves.

In Cuenca province, blood samples (n = 26) were collected immediately after death by cardiac puncture into EDTA-containing tubes. Haematological determinations were made in an automatic counter Technicon H-1. Differential cell counts were later confirmed from blood smears stained with Giemsa.

Collection of parasites

Heads were longitudinally opened and the cranial cavities examined for nematodes, which, when collected, were washed in physiological saline and fixed in 70% alcohol and cleared in lactophenol. Nematode identification was made according to Kutzer & Prosl (1975) and Demiaszkiewicz (1987). Extracting nematodes from lungs or muscle of the deer could not be undertaken.

Faecal samples of 10 g were obtained from the rectum of each deer and the Baermann technique was performed for the recovery of first-stage larvae. As it is difficult to distinguish first-stage larvae of *Elaphostrongylus* from other species of Protostrongylidae (Pybus *et al.*, 1989; Gajadhar *et al.*, 1994, 2000), only the dorsal-spined first-stage larvae longer than 390 μm were considered as *E. cervi* (Gajadhar *et al.*, 1994).

Data analysis

The prevalence of infection is expressed as the proportion of deer infected with *E. cervi*. The worm burden (intensity of infection) is expressed as the mean number of adult/preadult *E. cervi* per central nervous system (CNS). The production of the larvae in faeces of infected deer is expressed as the number of larvae per gram of faeces (lpg). Blood parameters measured were haematocrit (%), haemoglobin ($g\,dl^{-1}$), erythrocytes ($10^6\,mm^{-3}$), mean corpuscle volume (MCV, μ^3), mean corpuscular haemoglobin rate (MCH, pg), mean corpuscular haemoglobin concentration (MCHC, %), leucocytes ($10^3\,mm^{-3}$) and the percentage of neutrophils, lymphocytes, monocytes, eosinophils and basophils. Statistical differences in the prevalence and intensity of infection between host sex, infected or uninfected, sampling sites and season were tested using the Student's t, and χ^2 tests.

Results

Nervous system

Infection in the CNS could not be detected in deer examined from Toledo and Ciudad Real provinces. In Cuenca, *E. cervi* were found in the CNS of six (four females and two males) of 86 red deer (6.9%) with an intensity or mean worm burden of 3.8 nematodes per brain. All infected deer were captured in mid and late-winter when the prevalence of infection reached 50% (table 1).

Sampling sites clearly influenced the mean worm burden in deer, with infections in the Hosquillo National Park being statistically higher than in the Reserva Serranía de Cuenca ($P < 0.01$). Prevalence values from both sites were not statistically different although differences in the

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Table 1. Seasonal prevalence (%) and intensity of infection (mean number of worms per central nervous system) of *Elaphostrongylus cervi* in red deer from Cuenca, central Spain in 2000.

	Sampling site			
	Hosquillo National Park		Serranía de Cuenca	
Month	Prevalence (%)	Intensity	Prevalence (%)	Intensity
January	0.00	0.00	0.00	0.00
February	33.33	5.00	66.67	3.50
March	33.33	7.00	20.00	2.00
April–December	0.00	0.00	0.00	0.00

Table 2. Influence of management and host sex in the prevalence (%) and intensity of infection (mean worm burden of worms per central nervous system) of *Elaphostrongylus cervi* in red deer in a Continental mountain forest of central Spain.

	N	Prevalence (%) χ^2	Intensity ± SD (range) T-test
Management and sampling site			
12.42 deer km^{-2}			
controlled in a fenced reserve (Hosquillo National Park)	36	8.00	6.00 ± 1.41 (5–7)
3.70 deer km^{-2}			
totally free (Serranía de Cuenca)	50	5.56	2.75 ± 1.71 (1–5)
		ns	$P < 0.01$
Total	86	6.98	3.83 ± 2.23 (1–7)
Sex of deer*			
Males	31	6.45	4.00 ± 4.24 (1–7)
Females	52	7.69	3.75 ± 1.50 (2–5)
		ns	ns

*Data pooled from two sampling sites, sex was not determined in three animals.

prevalence and intensity of infection during the winter were statistically significant ($P < 0.01$) (table 2).

Four of the six infected deer were less than one year old and the remaining two deer were between one and two years old. Differences in the prevalence and intensity of infection between host sexes were not statistically significant.

No macroscopic lesions were found in the central nervous system in any of the deer examined.

Larval production

The production of dorsal-spined first-stage larvae in faeces was very low and irregular, with a maximum of 142 larvae per gram of faeces in June (table 3). The mean larval production was similar in both Continental (35.1 lpg) and Mediterranean areas (36.6 lpg). A total of 2373 first-stage larvae were measured but only ten (0.4%) were longer than the limit fixed to separate *E. cervi* larvae from those belonging other protostrongylid genera.

Table 3. Seasonality of protostrongylid first stage larval production in faeces of red deer from a Continental mountain forest area (Cuenca) and a Mediterranean tableland area (Toledo and Ciudad Real) in central Spain.

	No. of larvae per g faeces	
Month	Cuenca	Toledo and Ciudad Real
January	0.00	88.77
February	48.75	7.70
March	0.00	0.00
April	60.43	3.67
May	83.20	0.00
June	100.50	142.00
July	6.40	0.00
August	0.00	0.00
September	46.33	106.50
October	20.67	0.00
November	43.50	41.19
December	11.50	48.90

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Table 4. Blood parameters in red deer from Cuenca, central Spain, infected or uninfected with *Elaphostrongylus cervi*.

	Infected n = 5		Uninfected n = 21	
Blood parameter	Mean	SD	Mean	SD
Haematocrit (%)	47.72	4.17	41.38	8.44
Haemoglobin (g dl^{-1})	15.48	1.41	14.10	2.65
MCV (μ^3)	47.00	1.41	45.86	3.15
MCH (pg)	14.20	1.30	15.71	1.79
MCHC (%)	32.40	1.52	34.19	2.80
Erythrocytes $10^6\,mm^{-3}$	10.84	1.02	9.28	2.28
Platelets $10^3\,mm^{-3}$	181.40	211.58	172.05	120.19
Leucocytes $10^3\,mm^{-3}$	4.20	2.09	4.48	2.13
Neutrophils (%)	30.20		47.48	
Lymphocytes (%)	59.60		45.15	
Monocytes (%)	8.60		2.62	
Eosinophils (%)	1.60		4.48	
Basophils (%)	0.00		0.10	

No statistical differences in larval production were found in deer with or without worms in the brain.

Haematology

With the exception of lymphocyte and eosinophil numbers, the values of blood parameters (table 4) were higher than those reported previously from Spanish and European deer (Knox *et al.*, 1988; Soriguer *et al.*, 1994). No differences were observed between infected and uninfected red deer nor between host sex or age. White blood cells counts of red deer from the Hosquillo National Park (mean: $5.38 * 10^3\,mm^{-3}$, SD: 2.34) were higher than those in deer from the Reserva Serranía de Cuenca (mean: $3.61 * 10^3\,mm^{-3}$, SD: 1.47), differences that were statistically significant.

Discussion

Although the prevalence of *E. cervi* in red deer shows much variation, even between neighbouring municipalities (Stuve, 1986), the levels of infection in the CNS of deer in the present study is remarkably similar to that in other European areas like Russia (Blazek, 1976), Hungary (Sugar & Kavai, 1977), Switzerland (Hollands, 1985) Poland (Demiaszkiewicz, 1985, 1987) or Denmark (Eriksen *et al.*, 1989).

Unfortunately, the muscle, which is the main site of infection by adult worms of *E. cervi* (Prosl & Kutzer, 1980b; Hollands, 1985), was not available for examination and hence a direct comparison with previous work could not be made. Nevertheless, the number of worms per brain and the absence of macroscopic lesions in the meninges are consistent with the findings of previous authors (Hollands, 1985; Eriksen *et al.*, 1989).

Differences in the mean worm burdens between both reserves of Cuenca may be due to the deer stocking rate and to the movement of red deer in the Hosquillo National Park being more restricted than in the Reserva Serranía de Cuenca. The absence of infection in deer from Toledo and Ciudad Real, where the mean temperatures are high and the rainfall less than in Cuenca, could be due to climatic differences. In other colder and wetter areas in Europe, a rise in temperature from 11.8 to 12.7°C in summer is related to the presence of cerebrospinal disease during the following winter (Handeland & Slettbakk, 1995). In central Spain, even in Cuenca province, the summer temperature is usually higher (with a mean of 22.8°C being recorded from July to September during 1980 to 1998), but not too high to enable larval development to occur in the intermediate host. A drop in the mean temperature to 12.5°C during the summer 1999 in Cuenca was, however, sufficient for first-stage larvae to continue their development to the infective stage. Infection in deer is likely to have taken place in the summer of 1999, as specimens of *E. cervi* were detected in the brain during February–March 2000, once the prepatent period (107–125 days; Watson, 1983) had passed.

These results are in agreement with Hollands (1985) and Stuve (1987) who both indicated that winter is the main period for higher prevalences and intensities of infection. We only found *E. cervi* infection in the CNS of young deer and the fact that no infection was found during the spring to autumn is likely to be due to the location of *E. cervi* in the brain being a temporal developmental phase (Handeland *et al.*, 2000b). The level of infection declines when deer are 18 months old due to acquired immunity (Stuve, 1986, 1987).

The infection of *E. cervi* in males is higher than in females irrespective of age (Stuve, 1986). In the present study, there were no statistical differences between infection and host sex, probably because most of the deer examined were very young and sexual differentiation had not been completed.

Vicente & Gortazar (2001) recently reported a high prevalence (67%) of *E. cervi* larvae in the faeces of red deer from central Spain using the Baermann larval migration technique and these results are in agreement with Prosl & Kutzer (1980b) who found a yearly rhythm in larval production. However, the limitations of this technique for detecting first-stage larvae of *E. cervi* in faeces are well known (Pybus *et al.*, 1989; Gajadhar *et al.*, 1994, 2000) and the present results (0.4% of the larval production), indicate that the production of first-stage larvae of *E. cervi* in sites in central Spain is low overall and sporadic, as found in other areas (Gajadhar *et al.*, 1994; Gajadhar & Tessaro, 1995). Unfortunately, a direct comparison cannot be made with the work of Vicente & Gortázar (2001) as they found no lesions in the lungs which are the main lesion due to this nematode species (Luzón *et al.*, 2000) and they did not examine the CNS.

The blood characteristics of deer are influenced by diet, season, handling, high requirements during the rut, stress and other factors (Klinger *et al.*, 1986; Maede *et al.*, 1990; Bateson & Bradshaw, 1997). The high values of blood parameters in deer in the present study when compared with other Spanish and European deer (Knox *et al.*, 1988; Soriguer *et al.*, 1994) could be due to the method of capture, where general trauma and tissue damage may be caused by shooting the deer (Klinger *et al.*, 1986; Marco & Lavin, 1999).

Some aspects, such as the prevalence and intensity of infection, host age and the timing of infection suggest that *E. cervi* could be more widespread in Spain than is generally believed and its epidemiology is likely to be similar to that in other European countries where this

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nematode produces severe disease. Although the presence of large numbers of nematodes between the membranes of the brain and spinal cord may not produce nervous symptoms (Prosl & Kutzer, 1980a) it is important to remember that *E. cervi* does cause cerebrospinal disease in wild and domesticated ruminants (Borg, 1979; Lankester & Fong, 1998; Handeland & Slettback, 1995; Demiaszkiewicz *et al.*, 2000; Handeland *et al.*, 2000a). Furthermore, the occurrence of elaphostrongylosis in small ruminants is related to the presence of cervids in the area (Handeland & Slettbakk, 1995; Pusterla *et al.*, 1998a). Further studies on the ecology of *E. cervi* larvae in the intermediate snail hosts, together with the prevalence of adult worms in interconnective tissue and the seasonality of infection in deer and other definitive hosts, are now required.

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