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Occurrence, prevalence, and explanatory environmental variables of *Spirocerca vulpis* infestation in the foxes of western Spain

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Abstract

The main aim of this study was to not only establish the prevalence of the recently described *Spirocerca vulpis* parasite in the wild-life cycle of carnivores in western Spain but to also elaborate a model to explain the risk of infestation based on 16 topo-climatic and habitat variables. During the period from June 2016 to November 2017, 1644 carcasses of red foxes (*Vulpes vulpes*) and another 105 wild mammals, legally hunted or killed in car accidents, were analyzed. Parasitic nodules of *Spirocerca* were found in 6% of the foxes, and the molecular analyses established a homology of our samples with the species *S. vulpis*. There were no differences in the occurrence of the infestation between sexes, but there were differences in terms of age, such that infestation was proportionally more frequent among young individuals. In terms of temporality, a higher percentage of positive cases was observed during the late-autumn and winter months, especially between December and February. This study provides new data on the factors that predispose *S. vulpis* infection in the red fox. Model results indicate that a spatial pattern exists in the occurrence and prevalence of this species in the studied area (higher probabilities to the west), and that this pattern seems to mainly be associated with topo-climatic variables.

Keywords *Spirocerca vulpis* · Red fox · Explanatory factors · Dung beetles · Topo-climatic factors · Habitat factors

Introduction

Spirocerca lupi (Rudolphi 1809) is a nematode that affects animals of the Canidae family (Bailey et al. 1963; Lobetti 2000). Most research on this parasitosis has focused on the dog as the definitive host. However, there is a great lack of information on the epidemiology and pathogenicity of *S. lupi* in wild hosts, specifically in foxes. Recently, a new species, *Spirocerca vulpis* (Rojas et al. 2018a), was described from red foxes in Europe, which is morphologically and genetically different from *S. lupi* and produces stomach nodules in these hosts (Rojas et al. 2018a). Adults of *Spirocerca* are usually located in nodules in the thoracic wall of the esophagus. The females expel embryonated eggs that leave with the feces into the environment. These eggs are then ingested by dung beetles (Coleoptera Scarabaeidae and Geotrupidae), where they develop into infectious third-stage larvae (L3) until they are ingested by the definitive host (Bailey et al. 1963; Mukaratirwa et al. 2010). Other transport or paratenic hosts sometimes participate in the cycle (Chhabra and Singh, 1972).

Cases of infestation by *Spirocerca* have been reported worldwide (Bailey 1972; Van der Merwe et al. 2008; Dantas-Torres and Otranto 2014; Ferrantelli et al. 2010; Szafrańska et al. 2007; Popiołek et al. 2011). In Spain, previous studies have shown that *Spirocerca* is present in foxes and dogs with prevalence of up to 22.5% depending on the province (Criado-Fornelio et al. 2000; Martínez-Carrasco et al. 2007; Sanchis-Monsonís et al. 2019). In general, a greater incidence has been described in warm climates, leading us to suspect that certain topo-climatic and habitat factors may predispose hosts to these parasites. We examined a large number

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of fox carcasses from western-central Spain. The objective was to describe the occurrence and prevalence of spirocercosis in this area, to discriminate the species responsible, as well as to estimate climatic and habitat-associated variables to determine the most likely factors conditioning the progress of this parasitism in Iberian wild foxes.

Materials and methods

Study area

The study was conducted in 202 municipalities in five provinces of western Spain (Cáceres, Salamanca, Zamora, Ávila, and Valladolid). The total area covered by these municipalities is about 19,851 km^2, ranging geographically from 49.336 and 39.945 in latitude to − 8.842 and − 4.891 in longitude (Fig. 1).

Animal collection

From June 2016 to November 2017, a total of 1644 carcasses of red foxes (*Vulpes vulpes*) were subjected to necropsies where they were collected or, alternatively, at the parasitology laboratories of the Faculty of Veterinary Medicine at the University of Extremadura. The sampled foxes came from government-authorized hunting within legal guidelines and for recreational or commercial purposes or from deaths in traffic accidents. Of the 1644 analyzed foxes, 52.2% were males (*n* = 859), 47.4% were females (*n* = 779) and 6 could not be sexed. The approximate age of each of the foxes was determined according to the analysis of the dental formula (Sáenz de Buruaga et al. 2001), differences in the collection date of the corpse from the most probable date of birth taking into account that foxes in Spain have a unique breeding season during the spring (Voigt and MacDonald 1984; Zapata et al. 1997), and the external appearance of the fox based on researcher experience.

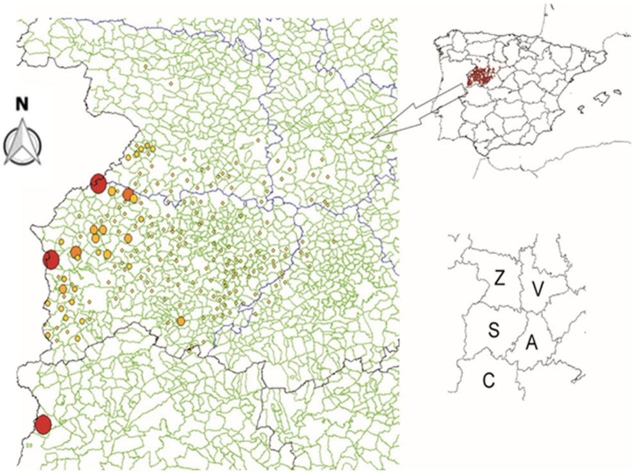

Fig. 1 Variation in the prevalence of *Spirocerca vulpis* derived from the foxes examined in 202 municipalities (green polygons) of peninsular Spain. Prevalence values varied from 0 (small yellow circles) to 1 (big red circles). The lower right map indicates the location of the Spanish provinces of Zamora (Z), Valladolid (V), Salamanca (S), Ávila (A), and Cáceres (C)

The corpses (*n* = 105) of nine additional mammal species killed in traffic accidents were also examined (see Table 1).

Determining *Spirocerca* infestation

The presence of parasitic nodules in the gastric wall or other locations during necropsy was considered the parameter of positivity of infection. No other parameter was considered to establish positivity, considering that coprological methods have not been highly effective (Al-Sabi et al. 2014).

The molecular study was carried out from a group of representative samples from the different geographical areas. DNA was extracted from individual specimens of worms corresponding to 31 *Spirocerca* spp.-positive red foxes using the kit Exgene Tissue SV (GeneAll, Seoul, Korea) following the manufacturer's instructions. PCR amplification was carried out as described previously for Spirurida (Casiraghi et al. 2001), where a 689 bp-long region of the *cox1* gene was amplified using the primer set NTF (5′-TGATTGGTGGTTTTGGTAA-3′) and NTR (5′-ATAAGTACGAGTATCAATATC-3′). All the amplicons were sequenced by Macrogen Sequencing Service (https://dna.macrogen.com), where for automated directing sequencing, the PCR products were amplified using the internal forward and reverse primer. Direct PCR sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle Sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice and then analyzed

Table 1 Number and taxonomic identity of the studied animals

	No. of animals of sampling
Negative red foxes sampled (*Vulpes vulpes*)	1544
Positive *Spirocerca vulpis* red foxes (*Vulpes vulpes*)	100
Genet (*Genetta genetta*)	9
European badger (*Meles meles*)	22
Marten (*Martes foina*)	29
Wild cat (*Felis silvestris silvestris*)	11
European polecat (*Mustela putorius*)	9
Hedgehog (*Erinaceus europaeus*)	4
Egyptian mongoose (*Herpestes ichneumon*)	7
Eurasian otter (*Lutra lutra*)	3
Wild boar (*Sus scrofa*)	11
Total	1749

by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). Sequences were edited in Chromas Lite 2.1.1 (Technelysium Pty Ltd) and consensus sequences for each forward/reverse pair were created in BioEdit (Hall 1999). The identity at the species level was assessed based on the analysis of the generated sequences, taking into consideration the higher results of homology searches using the sequences deposited at GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Origin of environmental data

Two types of environmental data, topo-climatic and habitat variables, were used, and their predictive capacity accounting for the occurrence and prevalence of *S. vulpis* infestations in the studied municipalities was assessed. In total, 11 topo-climatic variables were considered to measure the probable environmental degree of association of *S. vulpis* with climatic and topographic characteristics. Data on current climate came from the University of Extremadura (see methodology in Felicísimo et al. 2011), which include average figures about temperature and precipitation during the period from 1950 to 2007 for the complete Iberian Peninsula (see, http://ide.unex.es/conocimiento/). Using this primary source of climatic information at a resolution of 1 km^2 UTM grid cells and the formulas of Valencia-Barrera et al. (2002) and López-Fernández and López (2008), nine bioclimatic variables were built: mean monthly precipitation, minimum monthly precipitation, maximum monthly precipitation, total annual precipitation, minimum monthly temperature, maximum monthly temperature, mean monthly temperature, continentality, and aridity. Topographic data came from a digital elevation model of the Iberian Peninsula at a resolution of 90 m downloaded from the same Web page of the University of Extremadura. This topographic information was used to derive two variables: mean elevation and elevation range (difference between the minimum and maximum elevation in each municipality).

Habitat variables come from the CORINE Land Cover project for the year 2011 (www.eea.europa.eu) at a resolution of 100 m^2, using three of the five recognized land uses of level 1: natural (forest and semi-natural areas), cultivated (agricultural areas), and urban areas (artificial surfaces). Human population density of each municipality was also included as a habitat variable according to the data provided by the Instituto Nacional de Estadística (http://www.ine.es/). All of these four habitat variables were included in order to account for the possible effect of anthropic alterations in promoting or supressing this disease.

The obtained digital cartography was overlapped with the polygons of the 202 considered municipalities downloaded from the Instituto Geográfico Nacional (http://centrodedescargas.cnig.es). Average values of each of the 15 formerly mentioned variables were obtained for each municipality using GIS software (TerrSet 18.21; Clark Labs, Clark University).

Statistical analyses

The 1644 cases of foxes were first analyzed to examine if sex and age factors are associated with the occurrence of *S. vulpis*. A Chi-square test was used to estimate the association between the two binary variables (male/female sex and positive/negative infestation). In the case of age, 1-year classes were established, and the Wald-Wolfowitz non-parametric test (WW test) was calculated to compare the distribution of the number of observations in these age classes among fox specimens with and without *S. vulpis* infestation. The WW-test was used because it is not only based on the comparison of mean values, but also on the shape of the complete distribution of these values (Sprent and Smeeton 2007).

Subsequently, all the available data were used to determine the presence-absence of *S. vulpis* in each of the 202 municipalities as well as the prevalence of the infestation in each municipality (the number of positive cases on the total number of carcasses). These two dependent variables were regressed against the selected topo-climatic and habitat variables. Generalized Linear Models (GLMs) were used to quantify the relevance of each variable individually on either (i) the presence-absence of *S. vulpis* or (ii) the continuous variation in prevalence values. Thus, separate models were run to compare the effect of the predictors in delimiting the occurrence of the infestation (presence-absence data) as well as the frequency of the infestation in those municipalities with positive cases. For the first case, we used a binomial error distribution linked to the set of predictor variables via a logit link function. In the second case, a Poisson distribution was assumed and a logarithmic link function was used. The Wald statistic was used as a test of the significance of the regression coefficient, based on maximum likelihood estimates. In these analyses, land use frequencies were submitted to a logit transformation to fulfill linear modeling assumptions (Warton and Hui 2011), while the remaining predictors were standardized to eliminate the effect of measurement scale differences (to a mean of zero and standard deviation of one). Our aim with these models was simply to estimate the individual explanatory capacity of each variable, and this variability is measured as their change in deviance from a null model (Dobson and Barnett 2018).

The complete variability explained by each of the two types of predictors (topo-climatic (TC) and habitat variables (H)) was estimated submitting the data to a saturated model in which all the variables of each type were included together. The number of collected foxes in each municipality may influence the obtained prevalence values because the smaller the number of foxes, the greater the probability of obtaining negative cases, and thus low prevalence values. Consequently, the number of collected foxes in each municipality is also considered a third type of variable reflecting possible "sampling bias" (SB, hereafter). The comparative influence of TC, H, and SB variables in the occurrence or prevalence of *S. vulpis* infestations was estimated by a variation partitioning method or partial regression analysis (Legendre and Legendre 2012), where the complete explained variability is divided into eight different pure and combined components: three pure effects for each type of predictor, three combined variations between pairs of predictor types, one variation due to the combined effect of the three types of predictors and, finally, the variation not explained by the predictor variables included in the analysis. This analysis aims to discriminate the genuine relevance of each of the three types of variables, as well as their combined explanatory capacity as a consequence of the correlation between them (variability that can be indifferently attributed to one or the other set of predictors).

The variation of *S. vulpis* prevalence values between municipalities seems to show a clear spatially structured pattern (Fig.1). The weight of this spatial pattern was measured regressing prevalence values against the nine terms of a third degree polynomial equation of the central latitude and longitude of each municipality (trend surface analysis or TSA; Legendre and Legendre 2012). Similarly, after making a complete saturated model including all the TC, H and SB variables, the residuals (the variability unexplained by these predictors) were also submitted to a TSA to assess whether one or several important spatially structured explanatory variables could be left out (Legendre and Legendre 2012) or, on the contrary, whether the variables used represent this spatially structured pattern.

All statistics were performed using StatSoft's STATISTICA v10.0 (StatSoft Inc., Tulsa, Oklahoma, USA).

Results

The molecular analysis with BLASTn of 31 individual specimens of *Spirocerca* worms, always revealed homologies that oscillated between 99 and 100% with the cox1 sequences of *Spirocerca* sp. (examples of accession numbers: KJ605489.1 and KJ605487.1) and between 98 and 100% with *S. vulpis* (examples: MH634016.1 and MH633993.1), being the homologies always below 93% with respect to the sequences of *S. lupi*. Therefore, BLAST could allow a preliminary identification of our sequences with *S. vulpis*, recently described by Rojas et al. (2018a) for red fox, even though different phylogenetic and morphometric studies are necessary for greater robustness (work in progress).

The number of analyzed foxes throughout the year varied due to differences in the periods in which hunting is allowed in the study area (Table 2). The number of positive animals, as well as the percentage of positive cases, was higher during the winter months, especially between December and February (Table 2). The infestation of *S. vulpis* was observed in 100 red foxes with a general prevalence of 6.09%, which seems to vary spatially (Fig. 1). In terms of the provinces, Salamanca showed a prevalence value in foxes of 6.95%, while Ávila and Valladolid did not have any positive animals (Fig. 1). Only one fox was analized in the province of Cáceres and was positive, while a prevalence of 4.11% was obtained for the province of Zamora. The remaining mammal species were negative for the presence of *Spirocerca* spp. infestation.

The prevalence by sex reached 5.3% in males and 6.9% in females, but no significant association was observed (Chi-square = 1.77; $df = 1$; $p = 0.18$), such that the degree of infestation would be similar between males and females.

The number of analyzed foxes in which the age could be determined was 1637. Animals older than 1 year ($n = 1247$) had a prevalence of 5.8%, while young animals under 1 year ($n = 390$) reached a prevalence of 7.2%. In terms of age ranges, the highest number of positive cases was found in animals between 1 and 2 years old (Fig. 2). The frequency distribution of positive and negative cases of *S. vulpis* infestation differed significantly among the different age classes (WW-test = 17.99; $p < 0.001$; $n1 = 1537$; $n2 = 100$), mainly as a consequence of the proportionally lower number of positive cases in the older age classes (Fig. 2). Thus, infection was proportionally more frequent in young individuals.

Eight predictors were able to explain more than 10% of total variability in the occurrence of *S. vulpis* in municipalities (Table 3), highlighting among them topo-climatic variables such as mean elevation, minimum monthly temperature, and mean monthly temperature. In general, *S. vulpis* is more likely to be found in foxes when elevation is lower and minimum monthly temperatures are higher (Fig. 3). Considering habitat variables, the probability of finding *S. vulpis* is also higher in areas of natural land use (Fig. 3; Table 3). All topo-climatic predictors are able to account for 48.5% of total variability when considered together, while habitat variables taken together explain 15.6%. However, the occurrence of *S. vulpis* is also importantly accounted for by the number of collected foxes (SB variable; Table 3). Thus, *S. vulpis* is more likely to be found when the number of studied foxes is higher. A complete saturated model including all TC, H, and SB variables accounted for 59.8% of total variability. Partial regression analysis (Fig. 4) indicated that TC variables have the highest explanatory capacity independently of the other predictors (pure variability; 28.9%), and also that the pure effect of SB is not negligible (8.1%). A good part of the explained variability cannot be ascribed to any of the three types of predictors (11.3; i.e., combined effect of the three types of predictors). Finally, TSA analysis showed that the occurrence of *S. vulpis* is structured spatially; spatial variables explained 44.6% of total variability ($F = 17.14$; $df = 9, 192$; $p < 0.0001$). However, this spatial structure seems to be completely explained by the selected TC, H and SB predictors because the

Table 2 Number of collected foxes (*N*), number of foxes with positive infestation (N_{POS}) of *Spirocerca vulpis*, and percentage of infested foxes in the different seasons and months (winter (Win); spring (Spr); summer (Sum); autumn (Aut)) and months of the year from January (J) to December (D)

Seasons	Win	Win	Win/Spr	Spr	Spr	Spr/Sum	Sum	Sum	Sum/Aut	Aut	Aut	Aut/Win
Months	J	F	M	A	M	J	J	A	S	O	N	D
N	170	65	21	–	1	4	13	198	240	309	397	223
N_{POS}	16	13	0	–	0	0	0	6	9	8	26	22
%	9.4	20.0	0	–	0	0	0	3.0	3.8	2.6	6.5	9.9

residuals of the complete saturated model cannot be explained by a TSA ($F = 0.71$; $df = 9192$; $p = 0.70$).

Six predictors accounted for more than 10% of total variability of prevalence values between municipalities (Table 3). In this case, maximum monthly temperature, mean elevation and, especially, the number of collected foxes (≈ 46%) were the most relevant variables. Thus, as in the case of presence-absence data (occurrences), the prevalence of *S. vulpis* is higher at lower elevations and higher temperatures (Fig. 5). However, in the municipalities with positive cases, the prevalence of infection was higher when the number of collected foxes was lower (Fig. 5). In total, topo-climatic variables account for almost 64% of total variability in *S. vulpis* prevalence. However, habitat predictors only explain 6.8% when they are considered together. The number of collected foxes is in this case a very relevant predictor capable of accounting for almost 46% of total variability (Table 3). Thus, the higher prevalence of *S. vulpis* appears when the number of foxes in low (Fig. 5). A complete saturated model including all TC, H, and SB variables accounted for 86.2% of total variability. Partial regression analysis (Fig. 4) indicated that TC variables have the highest pure explanatory capacity (35.8%), and that the combined and inseparable effect of TC and SB variables is also high (32.7%); i.e., topo-climatic variables and the number of collected foxes covary in a way that makes it impossible to ascertain the explanatory capacity of each type of predictor. Interestingly, some of the combined effects are negative (Fig. 4), probably because the two types of variables together are able to explain the prevalence of *S. vulpis* better than the sum of their individual effects (Legendre and Legendre, 2012; a synergic effect). TSA analysis showed that the prevalence of *S. vulpis* is also structured spatially since spatial variables accounted for a large portion of total variability (62.4%; $F = 5.91$ $df = 9, 32$; $p < 0.0001$). Again, this spatial structure seems to be completely explained by the selected predictors; the residuals of the complete saturated model cannot be explained by a TSA ($F = 0.20$; $df = 9, 32$; $p = 0.99$).

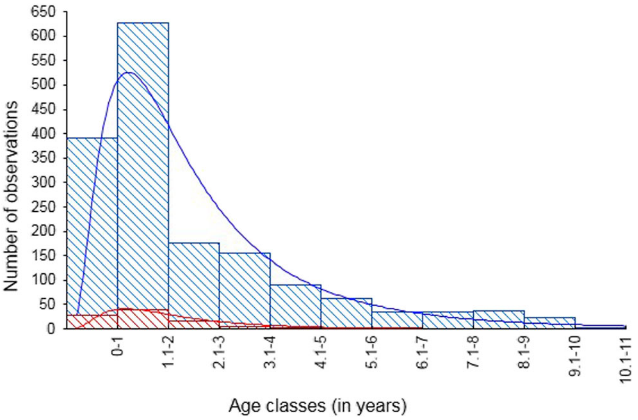

Fig. 2 Frequency distribution of the number of positive (in red) and negative (in blue) observations of *Spirocerca vulpis* infestation in foxes among different age classes. Lines represent the lognormal adjustment of the data

Discussion

Our study, which included 1644 foxes from different areas of western Spain, showed that *S. vulpis* has a prevalence of 6.09% in this region. The molecular analyses revealed a high homology of our sequences with the new species *Spirocerca vulpis*, described by Rojas et al. 2018a for red fox, confirming an apparent host preference to red foxes.

Therefore, this is the first study of variables and risk factors for the occurrence of *S. vulpis* in the west of Spain, thus getting better our understanding of their epidemiology and distribution in the Iberian Peninsula, although further phylogenetic and morphometric studies must be carried out to expand the knowledge about the evolution and phylogenetic variability within the genre, in the line with those presented by Rojas et al., 2018b and Sanchis-Monsonís et al. 2019.

Previous studies carried out in Spain indicate that the prevalence of up to 2.5% in the northeastern Iberian Peninsula (Gortázar 1997 and Segovia et al. 2004). In the Valencia Autonomous Community (eastern Spain), a study involving 286 foxes showed, however, a prevalence of 22% (Sanchis-Monsonís 2015; Sanchis-Monsonís et al. 2019). However, in the central part of the Iberian Peninsula the prevalence found reached 18.03% (for 67 foxes studied in the province of Guadalajara; see Criado-Fornelio et al. 2000) while any positive fox appears in other cases (for 61 foxes studied in the provinve of Ciudad Real; Valcárcel et al. 2018). In wolf populations of the Iberian northern plateau, *S. lupi* may have a prevalence of around 16% (Domínguez and de la Torre 2002).

Table 3 Deviance (Dev), percentage of explained deviance (% ExpDev), values of the Wald statistic measuring the statistical significance of the regression coefficient and associated probability of each of the considered predictors in accounting for the presence-absence of *Spirocerca vulpis* infestation (occurrence), and on the prevalence of *Spirocerca vulpis* infestation in foxes of the 202 studied municipalities

	Occurrence				Prevalence			
	Dev	% ExpDev	Wald stat	*p*	Dev	% ExpDev	Wald stat	*p*
Mean monthly precipitation	194.19	6.0	12.38	0.0004			0.38	ns
Minimum monthly precipitation	191.12	7.5	13.73	0.0002			0.28	ns
Maximum monthly precipitation	196.35	4.9	10.36	0.001			0.00	ns
Total annual precipitation	194.19	6.0	12.38	0.0004			0.38	ns
Minimum monthly temperature	139.05	32.7	51.65	≤0.0001			3.45	ns
Maximum monthly temperature	180.55	12.6	22.36	≤0.0001	5.89	27.6	13.49	0.0002
Mean monthly temperature	152.33	26.2	40.33	≤0.0001	7.05	13.4	5.22	0.02
Continentality	183.53	11.1	14.87	≤0.0001	7.08	13.0	4.91	0.03
Aridity	199.92	3.2	6.90	0.009			0.00	ns
Mean elevation	135.37	34.5	34.63	≤0.0001	6.14	24.6	14.29	0.0002
Elevation range			1.36	ns	7.15	12.2	5.06	0.02
Urban land use	201.85	2.3	4.57	0.03			0.04	ns
Cultivated land use	179.65	13.0	23.80	≤0.0001			0.24	ns
Natural land use	176.19	14.7	25.12	≤0.0001			0.01	ns
Population density			1.44	ns			0.48	ns
Number of collected foxes	165.10	20.1	27.79	≤0.0001	4.41	45.8	17.55	≤0.0001

In other southern European areas with similar climates, the prevalence found in foxes ranged from 9.16% to 23.5% in Italy (Ferrantelli et al. 2010; Magi et al. 2014) and 12.9% in Portugal (Eira et al. 2006). Taking into account all of these data, it is evident that the prevalence of *Spirocerca* spp. differs widely between Euromediterranean localities despite similar climates between zones and regions. This suggests that the influence of undetermined risk factors at the local level (local vegetatiton, water concentration, animal presence, kind of soil, human density population, etc.) may play a decisive role in the prevalence of this parasitosis.

In general, according to other authors, it seems clear that the factors that most affect the prevalence of *Spirocerca* spp. may be the presence of intermediate and paratenic hosts in the environment, as well as the density of definitive hosts infected in specific areas (Urquhart et al. 1996; Du Toit et al. 2008). In fact, in our study, despite having sampled areas with relatively similar global climatic characteristics, we found municipalities with prevalence values above 20% and others with no positive infestations. The density of intermediate hosts, such as dung beetles, in a given area may influence the variation in infestation prevalence, also considering that this density is related to other factors like soil substrate, climatic conditions, livestock density, and/or farming methods (Bailey 1972; Lumaret et al. 1992; Gottlieb et al. 2014). In fact, the higher prevalence values of *S. vulpis* were found in the dehesa areas with a high cattle density and also with high density of Iberian pig which eat acorns during autumn and winter (provinces of Salamanca and Zamora; see Fig. 1). In these areas, there is a greater availability of food sources for dung beetles, which would mean a greater density of these intermediate hosts, and in turn a greater probability of fox infections. This is in accordance with results provided by Frediani (1996), who found a greater percentage of dung beetles in the feces of foxes inhabiting pastures when compared with those inhabiting forest, which may be explained by the presence of a higher density of cattle in these pasturelands. The role of the dung beetle species colonizing livestock feces as intermediate hosts of *S. lupi* can be understood by considering that most part of Western Palearctic dung beetles are attracted to many different dung types (Martín-Piera and Lobo 1996; Frank et al. 2018). Thus, those natural areas with a high livestock density have a higher species richness, abundance and biomass of dung beetles (Lumaret et al. 1992; Galante et al. 1995; Lobo et al. 2006), and therefore, a greater probability of infection by *Spirocerca* spp. The results suggest that both the occurrence of *S. vulpis* infestations and the prevalence in the municipalities with positive cases seem to be spatially structured. Higher prevalence values and a high likelihood of obtaining positive fox infections occurred in the western zone of the studied territory on the border with Portugal. However, this spatial pattern disappears when climatic and habitat variables are considered, suggesting that an environmental component is at play in the infestation of *S. vulpis*. Some authors consider

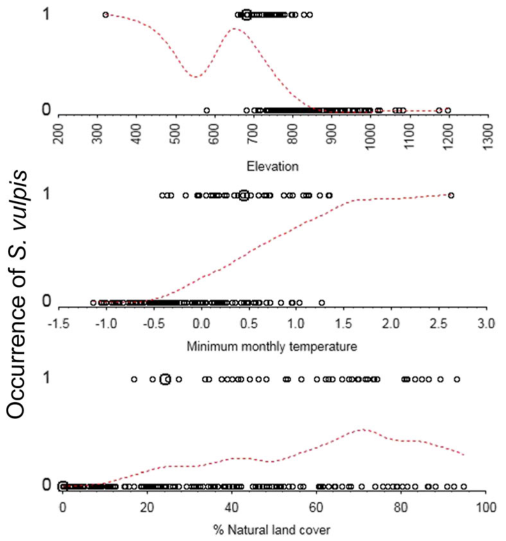

Fig. 3 Relationship between the negative (0) or positive (1) occurrence of *Spirocerca vulpis* in foxes and elevation, minimum monthly temperature, or percentage of natural land cover in the studied municipalities. The size of the dots indicates the number of observations. Broken line represents the adjustment of the data according to a distance-weighted least squares procedure

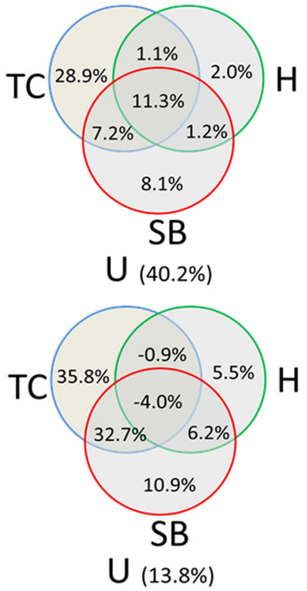

Fig. 4 Results of a partial regression analysis showing the pure and combined percentages of variability accounted for by topo-climatic (TC), habitat (H), and sampling bias (SB) variables on *Spirocerca vulpis* occurrence (upper diagram) and prevalence (lower diagram). U, unexplained variability. SB is the number of collected foxes in each municipality

that climatic variables, such as ambient temperature and humidity, may explain the rate of infection in the intermediate host. In previous studies with filarioid nematodes (Genchi et al. 2009; Laaksonen et al. 2010), high temperatures aggravated the infection of intermediate hosts. However, under more arid conditions, the risk of infection of *S. lupi* seems to be higher in irrigated and shady environments during summer (Gottlieb et al. 2011) and a decrease in humidity and an increase in maximum temperature seem to diminish the risk of *S. lupi* infections (Gottlieb et al. 2014). Our results indicate that climate variables have a greater explanatory power than habitat variables such that the occurrence, but mainly the prevalence of *S. vulpis*, is higher in lowlands and warmer regions. In our case, these lowlands are interspersed with trees and have the highest mean minimum and maximum temperatures. As with temperature, the decrease in humidity seems to cause a decrease in the infection rates of dung beetles (Gottlieb et al. 2014), coinciding with the conclusions drawn by Rojas et al. (2017). Interestingly, our results suggest that the role of the environment is greater in explaining the relative proportion of positive cases in the infected municipalities (≈ 64%), than in accounting for the presence-absence of *S. vulpis* (≈ 48%). This would suggest that the infestation of a region may depend on idiosyncratic local factors, but when a territory has been infested, the prevalence would be conditioned by environmental factors.

Our study also shows that the highest percentage of infection in foxes is found during winter months. These data are coincident with recent studies in the Iberian Peninsula, in a mediterranean climate area, where the highest probability of finding *S. vulpis* was at temperatures of 4 °C, decreasing well above 21 °C or below − 2 °C (Sanchis-Monsonís et al. 2019). In agreement with the results of previous studies carried out on dogs (Mazaki-Tovi et al. 2002; JyothiSree and Hafeez 2013), the seasonality of the occurrence of spirocercosis in the definitive host can be explained by the seasonality of the dung beetles themselves. According to the life cycle of the parasite, the highest incidence in foxes would occur between 3 and 6 months after the period of maximum activity of dung beetles (Van der Merwe et al. 2008). In our study area, it has been shown that the activity of dung beetles has two clear annual maximums as typical in Mediterranean regions: late spring and early autumn (Cabrero-Sañudo et al. 2008). This would imply that foxes become infected more frequently due to the ingestion of infected beetles occurring in the autumn season. This may occur for a variety of reasons, including an increased consumption of fruits and coleoptera by opportunistic foxes during early autumn (Carvalho and Gomes 2004; Santos et al. 2007; Díaz-Ruiz et al. 2011). This could also be due to the increased consumption of certain species of dung beetles depending on the season of the year. Assuming that some dung beetle species are more likely to act as intermediate hosts, additional decisive factors may also be the size of the beetle and the size of the particles they may consume (Serafini and Lovari 1993; Du Toit et al. 2012). More studies would be necessary to determine whether this is the case.

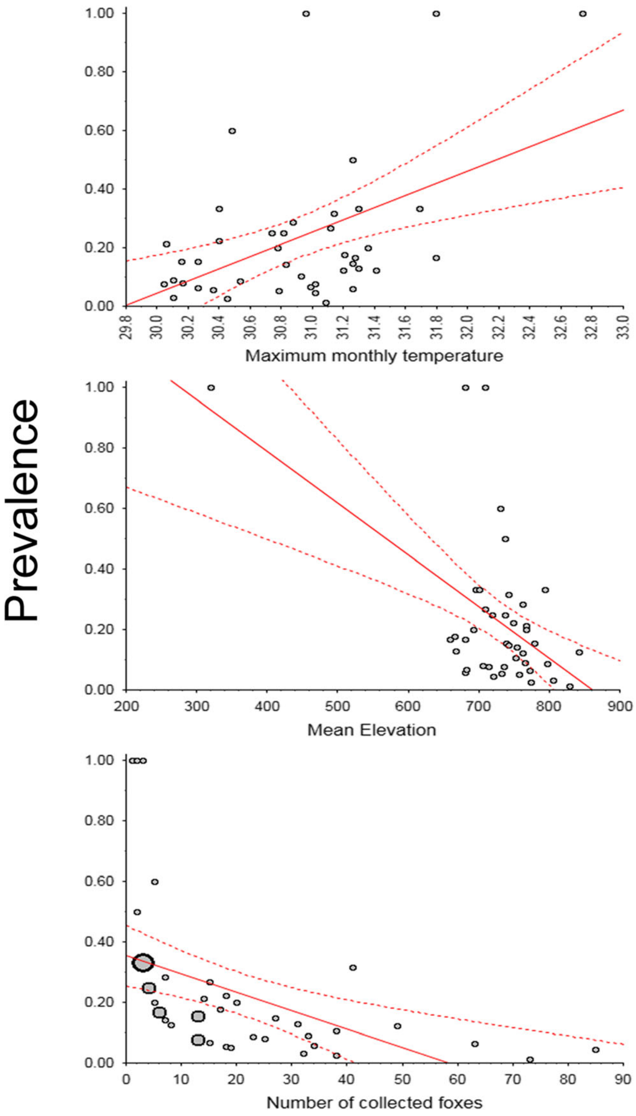

Fig. 5 Relationship between the prevalence of *Spirocerca vulpis* in the municipalities with positive cases and elevation, maximum monthly temperature, and number of collected foxes. The size of the dots depends of the number of observations. Solid lines represent the linear adjustment of the data and broken lines the 95% confidence interval

According to our results, there were no statistically significant differences in the occurrence of spirocercosis between males and females, as previously noted by other authors for foxes (Sanchis-Monsonís et al. 2019) and dogs (Wandera 1976; Mylonakis et al. 2006; Chikweto et al. 2012; Lobetti, 2014). Our results also show that fox age is relevant in explaining the presence of spirocercosis. The highest proportion of positive cases occurred in young foxes between 1 and 2 years old. At these ages, animals need a high protein intake and are more likely to actively look for insects such as dung beetles and other intermediate hosts of *Spirocerca* spp. (NRC 1982). Additionally, it is also likely that older animals have developed some degree of immunity due to repeated exposure to the parasite, as is the case in the infection of foxes by *Angyostrongylus vasorum* (Webster et al. 2017). Due to the absence of studies about the immune response of foxes to infection by *Spirocerca* spp., future work would be necessary to confirm this latter hypothesis. Sanchis-Monsonís et al. (2019) observed that the prevalence of spirocercosis in foxes was greater in animals older than 6 months than in offspring, a fact explained by the long life cycle of the worm. It is difficult to establish a clear comparison of this work with our results, since they considered animal adults from 6 months and we have established adulthood after the year of life, finding the highest rates of infection in animals between 1 and 2 years old.

In dogs, Lobetti (2000, 2014) did not identify a specific age group at risk for *S. lupi* infection; however, it has been reported that dogs between 1 and 4 years old are the most commonly affected (Chhabra and Singh 1972; Dixon and McCue 1967). Other studies carried out in dogs (Mazaki-Tovi et al. 2002; Aroch et al. 2015) found that animals 1-year old or younger had the lowest risk of spirocercosis infection. In this same species, the highest probability of infection occurs in animals of 5 or more years (Mazaki-Tovi et al. 2002; Sasani et al. 2012). When compared with our results, all of these studies seem to confirm that the age with the greatest likelihood of infection varies between regions and species.

In addition to the fox, our study about the prevalence of spirocercosis in western Spain included nine other wild mammals (see Table 1) in which the infection has not been detected in the performed necropsies. *S. lupi* is a nematode parasite of carnivores mainly found in Canidae, especially domestic dogs (*Canis familiaris*), although it has also been described in many domestic animals such as goats, ponies, donkeys, domestic cats, etc. (Ndiritu and Al-Sadi 1976; Mense et al. 1992). Similarly, in addition to the red fox, (Martínez et al. 1978; Diakou et al. 2012), spirocercosis has been reported in a wide variety of wild carnivores, including the coyote (*Canis latrans*), the gray fox (*Urocyon cinereoargenteus*), the bobcat (*Felis rufus*), the wolf (*Canis lupus*), the Neotropical bush dog (*Speothos venaticus*), the black-backed jackal (*Canis mesomelas*), the cheetah (*Acinonyx jubatus*), and lemurs (*Lemur fulvus*, *Lemur macaco*, *Lemur cattaurs*) (Murray et al. 1964; Blancou et al. 1976; Pence and Stone 1978; Domínguez and de la Torre 2002; Szczęsna and Popiołek 2007; Rinas et al. 2009; Bumby et al. 2017). We do not rule out the possiblity that a larger sample of these other wild mammals would yield positive cases of spirocercosis in the studied area.

Our results also provide some methodological implications associated with the role of the number of studied foxes in each municipality in explaining the occurrence and prevalence of spirocercosis. Because there was a greater likelihood of identifying positive cases of infestation when more foxes were tested, detecting infestation should require extensive sampling. Consequently, concluding that a municipality is clear of infection is risky due to the low infection prevalence (6%). Furthermore, when only the data from those municipalities with positive cases are considered, the prevalence is higher if the number of foxes collected is low. This result may also be a consequence of sampling biases. However, if the number of foxes captured in each municipality is positively related to their population size, this result may suggest that spirocercsis could be the one of the culprits behind the decrease in the number of foxes in the infected municipalities, but it cannot be ruled out the influence of other possible pathogens (viruses, other parasites, etc.).

We have not included domestic dogs in our study althought the risk of transmission of *S. vulpis* from foxes to domestic dogs should be viewed as high. However, Spanish legislation obligates to carry out deworming programs in dogs every 3–6 months thus hindering the spread of a disease requiring such a long time to complete its epidemiological cycle. Despite this, possible changes in this legislation, the gradual rapprochement of foxes to anthropized areas, together with the increasing of dog populations should be considered risk factors specific requiring monitoring programms in order to prevent the transmission of *S. vulpis* to dogs.

In conclusion, Spirocercosis is an important parasitosis in the red foxes of Western Spain, being the subspecies *S. vulpis* the main cause of this infection. The occurrence and prevalence of the infection seem to be determined by multifactorial causes, including the season of year, the age of the foxes, and especially, topo-climatic and habitat factors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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