






Predictive factors for flea occurrence in red foxes (*Vulpes vulpes*) from semi-arid Mediterranean environments

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Abstract

The role of red fox as host for a wide range of parasites, particularly fleas and other arthropods causing vector-borne diseases, in combination with its capability to adapt to anthropized environments, makes this wild canid an epidemiologically remarkable species at the wildlife–domestic–human interface, especially in the present time of rise of emerging and re-emerging diseases. This study evaluated the prevalence and parasite intensity of fleas in 88 foxes from Murcia Region (Southeastern Spain) and determined the geographic distribution of areas with the highest potential risk of flea presence. *Pulex irritans*, *Ctenocephalides felis*, *Spilopsyllus cuniculi* and *Nosopsyllus fasciatus* were identified. The overall prevalence was 76.13%. This is the first time that *N. fasciatus* has been reported in foxes from Murcia Region. The predictive model established a certain pattern to determine the areas with the highest risk of acquiring fleas. Positive correlation of daily potential evapotranspiration (ET_0) in winter and the opposite effect occurring for ET_0 in summer were obtained, as well as positive correlations for mean daily temperature (T_{mean}) in summer and mean precipitation (P_{mean}) in winter and summer. The model was also found positively correlated in the forest habitat ecotone areas and the anthropized areas.

KEYWORDS

ecotone, fleas, GIS, red fox, semi-arid Mediterranean environments, Spain

INTRODUCTION

Fragmentation and habitat loss due to human activity can cause an alteration in mesocarnivore communities (Recio et al., 2015). These changes are particularly striking in the Mediterranean environments of the Iberian Peninsula, where in a few decades, there has been significant soil degradation caused by land use and land cover changes in rural areas (Van Leeuwen et al., 2019). In this sense, the greater availability of trophic resources associated to an increase in anthropized

environments may facilitate the displacement of specialist species by generalist species and, consequently, changes in intra- and interspecific ecological interactions (Díaz-Ruiz et al., 2013).

Red fox (*Vulpes vulpes* Linnaeus, 1758) is one of the most widely distributed wild carnivores in the world and the most abundant carnivore in Southeastern Spain (Martínez-Carrasco et al., 2007). This wild canid is a generalist predator, with a broad trophic spectra and high ecological plasticity (Díaz-Ruiz et al., 2013), which has enabled it to adapt successfully to periurban environments in Spain. In fact, foxes

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prefer fragmented and heterogeneous habitats with different ecotones (Gloor et al., 2001).

The fox is host for a wide range of parasites, bacteria and viruses, many of them with relevant implications for human and animal health (Foley et al., 2017; Karamon et al., 2018). Its adaptation to anthropized environments, where domestic and wild synanthropic species are abundant, makes the fox an epidemiologically key species at the wildlife–domestic–human interface (Dáttilo et al., 2020). This fact, together with the participation of the fox in the maintenance of parasites with direct and indirect life cycles, the trophic habit of the species and the connectivity between wild and peridomestic ecosystems, resulting from the broad spatial ecology of the red fox, may be determinant factors of parasite transmission and, also, spillover risk (Foley et al., 2017).

Insect and arachnid ectoparasites are among the most ubiquitous fox parasites (Dáttilo et al., 2020). A relevant characteristic of these arthropods is their role as vectors of a wide variety of pathogens. After mosquitoes, ticks and fleas are the most important parasites as carriers of vector-borne pathogens (Bitam et al., 2010).

Fleas (Order Siphonaptera) are highly specialized hematophagous obligate parasites (Bitam et al., 2010; Whiting et al., 2008). Although the degree of coevolution with the host determines the specificity of many parasite species, fleas are rarely host species-specific (Bitam et al., 2010; Poulin et al., 2008). In general, hosts that use dens and are gregarious species typically have flea species adapted to their specific host. In addition, many flea species are adapted to a wide range of hosts (Poulin et al., 2006), such as *Pulex irritans* and *Ctenocephalides felis* (Bitam et al., 2010), and accidental parasite–host associations appear to be more common in fleas than other ectoparasites; for example, it is not uncommon to find the rabbit flea *Spilopsyllus cuniculi* on foxes, which often prey on this lagomorph (Whiting et al., 2008).

Ecological generalist species such as the red fox, host for a wide range of ectoparasites, could be used as sentinel species to identify areas of potential risk for vector-borne disease transmission (Aguirre, 2009; Foley et al., 2017). In this regard, fleas are one of the most abundant ectoparasites in foxes throughout the Palearctic region (Dobler & Pfeffer, 2011; Foley et al., 2017; Víchová et al., 2018). This, added to the high ecological plasticity of the fox, makes this wild canid a host species whose potential epidemiological role in vector-borne disease transmission must be taken into consideration, especially in anthropized areas. In this context, the importance of emerging and re-emerging diseases and the role played by wildlife as reservoirs should be emphasized (Daszak et al., 2000; El Hamzaoui et al., 2020; Hassell et al., 2017). A clear example is the current pandemic caused by the SARS-CoV-2 virus, which has highlighted the lack of information on the complex ecological host–parasite interaction networks (Dáttilo et al., 2020) and, consequently, the need to approach the study of diseases from a one-health perspective. In this sense, it has recently been suggested the possibility of SARS-CoV-2 transmission by fleas (Villar et al., 2020), highlighting the importance of research on these ectoparasites at the wildlife–domestic–human interface. As far as wild mesocarnivores are concerned, unfortunately, only a few studies have been carried out to date. This lack of information on wild carnivore

fleas may be due to the difficulty in obtaining live or recently dead animals, which is not always viable in wild carnivores due to their elusive habits; in most cases, the study is carried out on carcasses of hosts that have dead for several hours, which reduces the presence of fleas because they are highly mobile parasites that leave the host carcass within a short time (Nelder & Reeves, 2005).

The main objectives of this study are: (1) to study the richness and prevalence of flea species of red foxes from semi-arid Mediterranean environments subjected to different levels of anthropization; (2) to analyse the correlation between prevalence and parasite intensity with environmental and host-dependent factors; and (3) to define, using spatial analysis, the areas where the presence of foxes parasitized by fleas can be considered potential epidemiological hotspots for vector-borne diseases.

MATERIAL AND METHODS

Study area

The study was performed in Murcia Region (Southeast Spain), an area characterized by a Mediterranean subtropical climate (Figure 1). Its altitudinal heterogeneity (0–2000 m) and the influence of the sea are responsible for the presence of most of the bioclimatic zones of the Mediterranean region, with six different bioclimatic levels and three ombroclimates, being dominant the semi-arid ombroclimate, with rainfall ranging from 300 to 600 mm per year, in addition to a high annual potential evapotranspiration that ranges between 800 and 1300 mm (Hein, 2007). Traditional agroforestry ecosystems, although increasingly threatened due to low productivity and socioeconomic changes, still have an important presence (Van Leeuwen et al., 2019), providing potential habitats for mesocarnivores.

Sampled animals

A total of 88 foxes (54 males and 34 females) were sampled between April 2019 and April 2021. The seasonal distribution of sampling was 7 foxes in spring, 8 in summer, 25 in autumn, and 48 in winter. Foxes were road-killed animals throughout the road network of Murcia Region. Each intact carcass was placed in a plastic bag that had been previously sprayed with pirethrin to remove the ectoparasites present in the fox and was immediately carried to the Faculty Members of Veterinary (Murcia, Spain). To ensure that the fox had deceased recently, the carcass was examined to evaluate the algor mortis and to determine if rigour mortis was established. Typically, the onset of rigour mortis begins at approximately 2–6 h after death (Brooks, 2016). In most cases, rigour mortis appeared when the carcass arrived at the laboratory, indicating that the animal had just died. In some cases, when the researchers were unable to collect the carcass quickly, road maintenance technicians gathered the recently dead fox and kept it frozen until it was collected for inclusion in this study. Each fox was located by geographic coordinates (UTM) and correctly identified. The

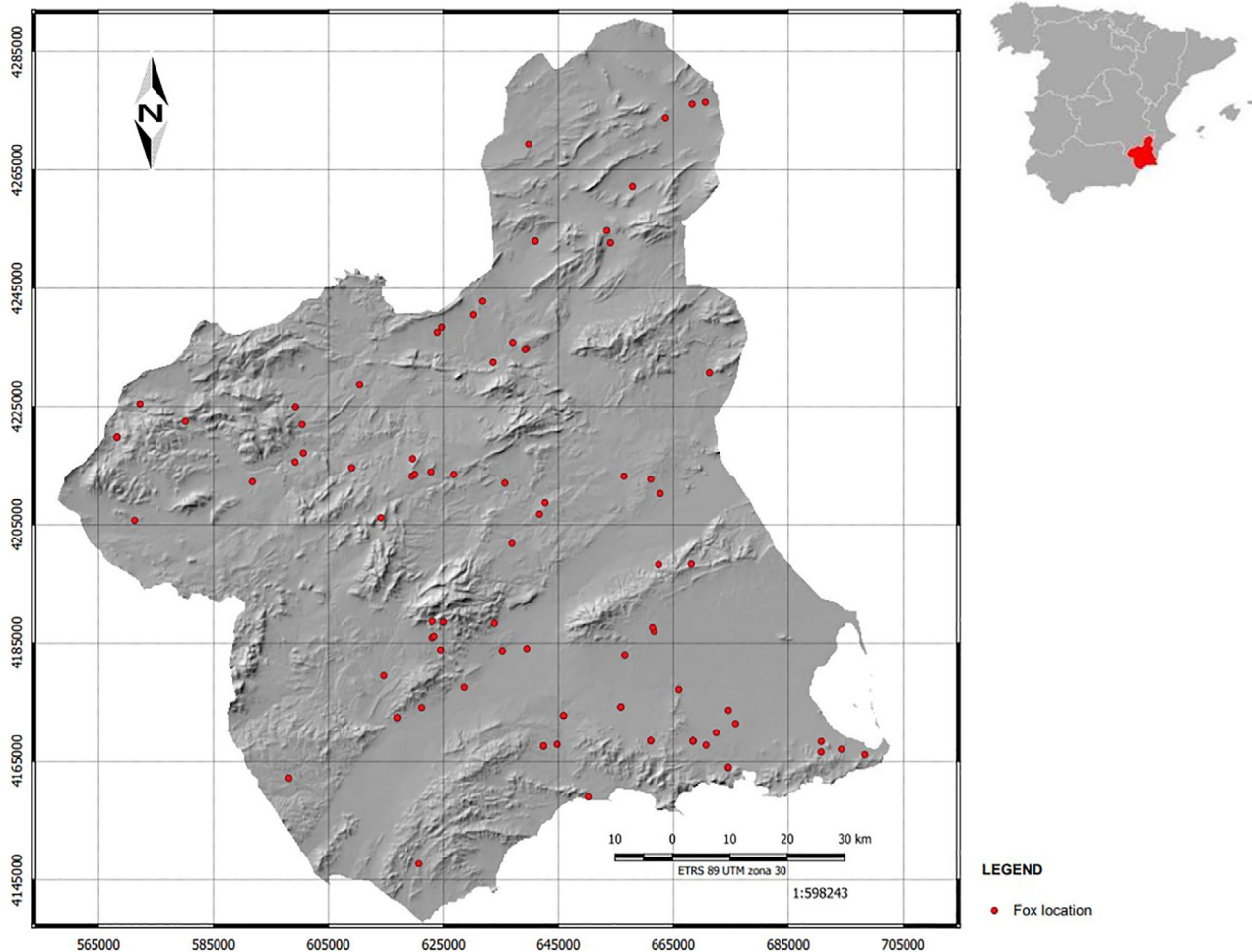


FIGURE 1 Geographical distribution of the foxes studied in Murcia Region (SE Spain). Red dots indicate the place of origin of each fox ($N = 88$)

following data were recorded: date of death, sex, age and weight (kg). Fox age was calculated according to Roulichová and Anděra (2007), classifying individuals as 5 juveniles (1–12 months old), 12 subadults (12–24 months old), and 71 adults (more than 24 months old). Moreover, to assess body condition, the general appearance of the animal was evaluated, paying special attention to muscle mass around lumbar vertebrae; specifically, five subjective categories (1–5) were established, being 1 the value assigned to a cachectic fox and 5 the value given to a fox with an excellent body condition.

Collection and identification of fleas

Immediately after the fox carcass arrived at the laboratory, the fur was brushed and carefully checked for the presence of ectoparasites for at least 10 min. All detected fleas were collected and stored in 70% ethanol until further preparation. Before identification, the specimens were processed by digestion and mounted in permanent preparations. Specifically, fleas were transferred to 8% acetic acid for 24 h.

Then, specimens were placed in distilled water for 1 h to stop digestion and, subsequently, mounted on Hoyer's medium; finally, the samples were incubated at 40°C for 48 h. Fleas were examined by optical microscopy, and species identification was made based on morphological features described by Smit (1957), Lewis (1967), and Linardi and Santos (2012).

Environmental factors

The environmental variables analysed were grouped into three datasets. The first set describes the climatic features of the study area. For this purpose, we extracted in raster layers from the digital climatic atlas of the Iberian Peninsula (Ninyerola et al., 2005) the average monthly and annual values of mean, minimum and maximum temperature, as well as radiation and precipitation. From the temperature and radiation values, the monthly and annual average values of ET_0 (potential evapotranspiration) were calculated following the Hargreaves formula (Hargreaves & Samani, 1985). The calculation was made using the simplified formula:

$$ET_0 = 0.0023 * (T_{\text{mean}} + 17.78) * R_0 * (T_{\text{max}} - T_{\text{min}})^{0.5}$$

where ET_0 is the daily potential evapotranspiration (mm/day), T_{mean} is the mean daily temperature ($^{\circ}\text{C}$), R_0 is the extraterrestrial solar radiation (mm/day), T_{max} is the maximum daily temperature ($^{\circ}\text{C}$), and T_{min} is the minimum daily temperature ($^{\circ}\text{C}$).

The second dataset is related to the spatial distribution of vegetation cover and soil moisture. This information was obtained from the reflectance values of the land surface from the OLI and TIRS sensors of the Landsat 8 satellite (USGS, 2021). To determine the seasonal changes in both parameters, two image acquisition dates were established to characterize the summer and winter periods (05/08/2019 and 12/01/2020, respectively), a period coinciding with the collection of the analysed foxes. Scenes of the study area were obtained from the USGS product service (2021). The downloaded satellite image was pre-processed at L1TP level with radiometric calibration and orthorectification. Conversion of the imagery to TOA reflectance and brightness temperature was performed with a semi-automatic classification plugin (Congedo, 2020) using QGIS (3.4.10). Vegetation and soil moisture indices were selected to characterize the most suitable environments for flea species. The normalized vegetation index (NDVI) is based on the ratio of the wavelengths of the visible spectrum in the red range ρ_r (0.64–0.67 μm) and in the near infrared ρ_{NIR} (0.85–0.88 μm) related to the photosynthetic activity of plants. The calculation formula is the following: $\text{NDVI} = (\rho_{\text{NIR}} - \rho_r) / (\rho_{\text{NIR}} + \rho_r)$. The normalized moisture index (NDMI) is determined from the wavelengths of the near infrared, and the Short Wave Infrared ρ_{SWIR1} (1.57–1.65 μm), according to the following equation: $\text{NDMI} = (\rho_{\text{NIR}} - \rho_{\text{SWIR1}}) / (\rho_{\text{NIR}} + \rho_{\text{SWIR1}})$. To complete the environmental characterization from satellite data, the Bare Soil Index (BSI) was obtained. The algorithm uses the wavelengths mentioned above in addition to the blue range ρ_b (0.45–0.51 μm) ρ_b of the visible spectrum, as described in equation: $\text{BSI} = (\rho_{\text{SWIR1}} + \rho_r) - (\rho_{\text{NIR}} + \rho_b) / (\rho_{\text{SWIR1}} + \rho_r) + (\rho_{\text{NIR}} + \rho_b)$. Finally, from the TIRS sensor, the thermal infrared band ρ_{TIR1} (10.60–11.19 μm) which estimates soil moisture and thermal mapping is applied.

The third dataset is formed by land cover types and land use. This factor is relevant for epidemiological studies on wildlife, since the segmentation of a territory subjected to different land uses affects the spatial distribution and movement patterns of wild species and, consequently, also influences the maintenance and dispersion of pathogens (Ostfeld et al., 2005). As a main source, the CORINE Land Cover project provides land cover data in the latest update (EEA, 2018). In addition, the classification, mapping and evaluation of the pasture resources of Murcia Region were used (Alcaraz Ariza et al., 2006).

Statistical analysis

Statistical significance tests for a 95%–94% CI were performed with R (R Core Team, 2020), and differences were considered significant when $p < 0.05$. Three datasets were analysed: (1) Fisher's exact test was used to evaluate the prevalence of fleas in relation to the different intrinsic variables of the host (sex, age, and body condition).

Parasite intensity (i.e., number of fleas per parasitized host) was evaluated for the same categorical variables using the Kruskal-Wallis Rank test; (2) Correlation between flea intensity and the following environmental variables was evaluated: temperature, precipitation, radiation, evapotranspiration, and soil surface temperature (using annual mean values); spectral indices for vegetation cover, bare surface, and humidity (mean values for summer and winter); land use, types of vegetation, and bioclimatic levels (percentage of cover occupied); and livestock rate (MJ/ha/year for bioclimatic level) (Alcaraz Ariza et al., 2006). Based on estimates of the home range for red fox (Deak et al., 2020), environmental variables were averaged for a 1 km buffer (400 ha) from the geographic location of each fox using QGIS (3.4.10) and Spearman Rank Correlation was tested for each predictor. (3) Exploratory Factor Analysis (EFA) was performed to determine which environmental variables, that were found to be statistically significant ($p < 0.05$), were grouped together to reduce the number of factors to be included in the model. The data extraction method applied was "maximum likelihood," in combination with the "promax" rotation method. To validate variables, Bartlett's test of sphericity (p value less than 0.001) and Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy (item values greater than 0.6) were used. Both tests are important to the extent that Bartlett's test compares correlation matrix (Pearson correlations) to check if there is a redundancy between variables that can be summarized with some factors. GLM models (Family = Poisson) were tested to evaluate parasite intensity as a function of predictor variables selected, and a model selection was performed using Akaike's Information Criterion (AIC) and the Deviance explained by each model.

The map of potential risk areas was generated using QGIS (3.4.10) (QGIS Development Team, 2021).

RESULTS

In our study, most of the 88 foxes had a body condition higher than 3 on the subjective scale used (36 foxes classified as category 3, 31 as category 4, and 8 individuals as category 5), 11 were clearly thin (category 2), and only two foxes were classified as category 1 (cachectic individuals).

The overall prevalence of fleas was 76.13% (67/88 foxes), and the parasite intensity ranged from 1 to 841 fleas with a mean value of 42.13 (± 112.87 SD) fleas per parasitized fox. The total number of fleas isolated was 2823. The most prevalent flea species was *Pulex irritans* (Linnaeus, 1758) followed by *Ctenocephalides felis* (Bouché, 1835), *Spilopsyllus cuniculi* (Dale, 1878), and finally *Nosopsyllus fasciatus* (Bosc, 1800) (Figure 2); in particular, prevalences were 75% (66/88 foxes), 35.2% (31/88), 19.3% (17/88), and 6.8% (6/88), respectively.

In terms of flea co-infections, 44.8% of the parasitized foxes had only one flea species (30/67), 35.8% had two flea species (24/67), 14.9% harboured three species (10/67), and 4.5% of foxes had coinfection by four species (3/67).

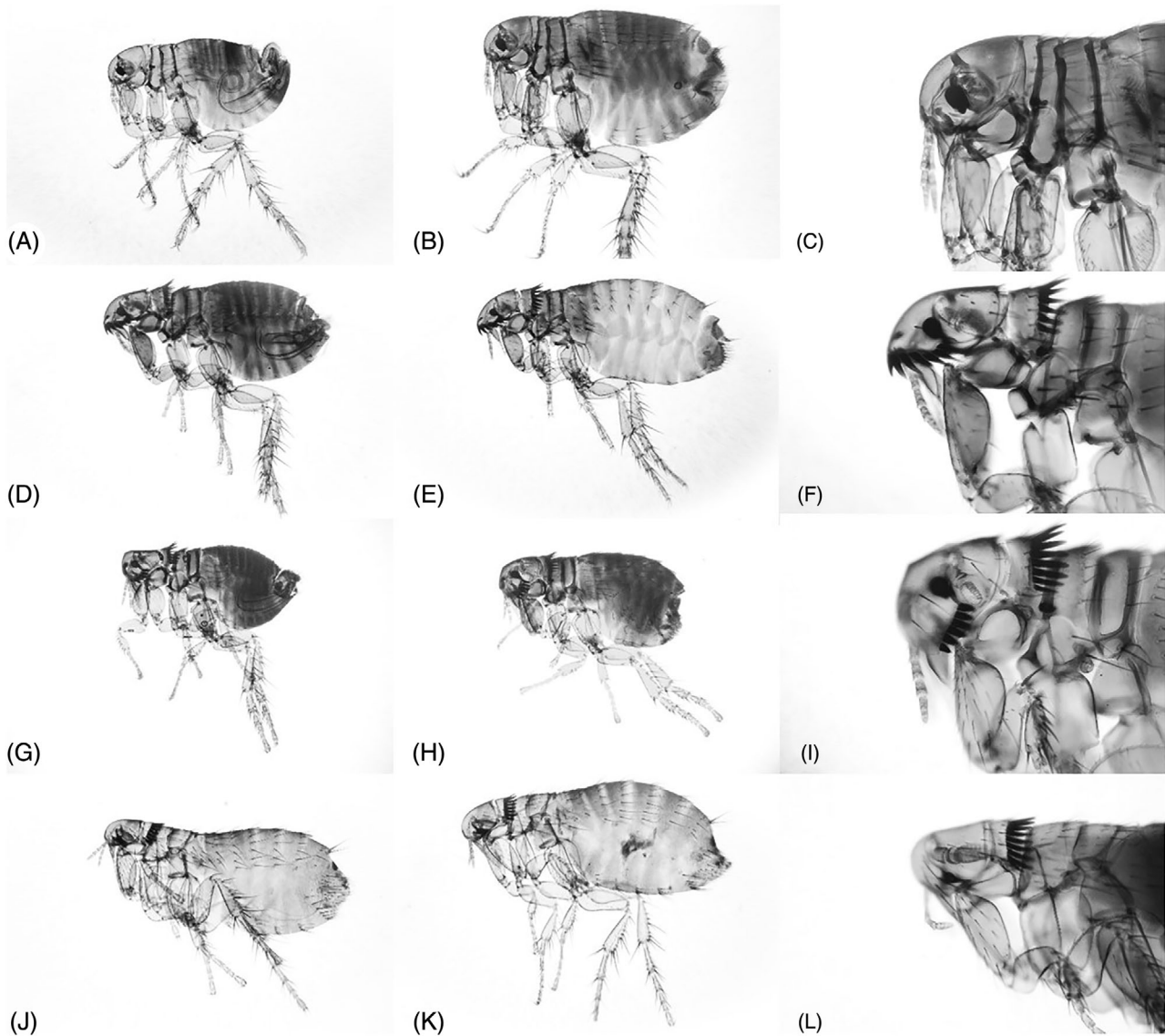


FIGURE 2 Flea species identified: a) *Pulex irritans* (δ); b) *P. irritans* (φ); c) *P. irritans*, head detail; d) *Ctenocephalides felis* (δ); e) *Ct. Felis* (φ); f) *Ct. Felis*, head detail; g) *Spillopsyllus cuniculi* (δ); h) *S. cuniculi* (φ); i) *S. cuniculi*, head detail; j) *Nosopsyllus fasciatus* (δ); k) *N. fasciatus* (φ); and l) *N. fasciatus*, head detail. $4\times$, $10\times$ optical microscope. All photos at different scales

According to the first dataset analysed (1), the prevalence of fleas did not differ significantly between sex, age, and body condition categories (p -values = 0.0709, 0.4948, and 0.7805, respectively). For the parasite intensity, sex was the only statistically significant category for a 94% CI (p -value = 0.0547**) with non-significant values for the rest of categories (p -value = 0.3235 and 0.1080, respectively) (Table 1, Figure 3).

Second dataset analysis (2) determined that the statistically significant environmental variables were: T_{mean} May, T_{mean} June, T_{mean} Aug, P_{mean} Anual, P_{mean} Jan, P_{mean} Feb, P_{mean} Mar, P_{mean} May, ET_0 Anual, ET_0 Jan, ET_0 Feb, ET_0 Mar, ET_0 Apr, ET_0 May, ET_0 Jun, ET_0 Sep, ET_0 Oct, ET_0 Nov, ET_0 Dec, T_{mean} Surface summer, Upper Termomediterranean, Bare Surface summer, Corine₁₃₃ (Construction sites), Corine₁₄₁ (Green urban areas), Corine₂₁₂ (Permanent irrigated land), Corine₃₁₂ (Coniferous forest), Corine₃₂₃ (Sclerophyllous vegetation),

Vegetation Type₁ (Dense trees), Vegetation Type₂ (Cereals), and Livestock rate (Table S2, supplementary material).

In the third dataset (3), EFA determined that the statistically significant variables were grouped in 3 factors that explained 26.6%, 22.7%, and 22.7% of the dataset variance, respectively. The environmental variables with the highest statistical significance within each factor were: Factor 1 (ET_0 Jun, P_{mean} Jun, T_{mean} Surface summer); Factor 2 (T_{mean} May, P_{mean} May, Upper Termomediterranean); and Factor 3 (ET_0 Dec) (Table S3, supplementary material).

The predictive model generated explained 25.47% of the data variance. The variable that most influenced the model was ET_0 in winter, followed by ET_0 in summer, with a positive correlation in the first case and a negative correlation in the second, that is, higher ET_0 in winter determines increased flea intensity, and a higher ET_0 in summer determines a lower one. It should be noted that ET_0 is

TABLE 1 Overall prevalence and intensity of flea species in foxes from Murcia Region (SE Spain), according to the sex, age, and body condition (BC) of the host (N = 88). M: Male, F: Female; J: Juveniles, S: Subadults, A: Adults; 1: Cachectic, 2: Thin, 3: Good (BC), 4: Very good BC, 5: Excellent BC.

Species	Fox category (prevalence n/N %)													
	Positive foxes					Body condition								
	Prevalence		Intensity			Sex		Age						
	n	%	Maximum per fox	Mean (±SD)	M (54)	F (34)	J (5)	S (12)	A (71)	1 (2)	2 (11)	3 (36)	4 (31)	5 (8)
<i>P. irritans</i>	66	75	792	39.04 ± 107.22	81.48	64.70	100	83.33	71.83	50	63.64	77.77	74.19	87.50
<i>C. felis</i>	31	35.23	42	2.48 ± 7.46	42.59	23.53	20	66.66	30.98	0	36.36	25	45.16	50
<i>S. cuniculi</i>	17	19.32	15	0.66 ± 2.05	20.37	17.48	0	16.66	21.13	0	9.09	11.11	29.03	37.50
<i>N. fasciatus</i>	6	6.82	2	0.12 ± 0.40	9.26	2.94	0	8.33	7.04	0	0	2.77	12.90	12.50
Total	67	76.13	841	42.13 ± 112.87	83.33	64.70	100	83.33	73.24	50	72.73	77.77	74.19	87.50
p-value (Prevalence /Intensity)	-	-	-	-	0.0709/0.0547**	-	0.4948 / 0.3235	-	-	-	-	-	-	-

Note: Statistical significance tests for a 95%-94% CI, and differences were considered significant when p-value <0.05 * and <0.06 **. Total number of fleas isolated: 2823.

determined by temperature and humidity; therefore, in arid and semi-arid environments, higher ET_0 values indicate higher temperature and lower humidity. The second most important variable was T_{mean} in summer, with a positive correlation to parasite intensity, that is, higher temperature in summer determines higher flea intensity. Finally, P_{mean} was the variable with the least influence on the model, with a positive correlation for both winter and summer. In relation to Corine landcover, the categories with higher influence in the model were Corine₃₁₂ and Corine₃₂₃, with a positive correlation in the first case and negative for the second, indicating higher flea intensity in coniferous forest areas and lower in zones with sclerophyllous vegetation. For the remaining categories, Corine₁₃₃ was positively correlated and Corine₂₁₂ was inversely correlated, indicating higher flea intensity in urbanized areas and lower in permanently irrigated areas.

The potential risk areas for acquiring fleas were estimated according to the following linkage equation obtained in the selected GLM model:

$$\text{Number of fleas} = 10^{(-1.720e^{(+01)} - 1.872e^{(-02)}(ET_{0,mean} \text{ June}) + 4.500e^{(-01)}(ET_{0,mean} \text{ December}) + 5.165e^{(-03)}(P_{mean} \text{ May}) + 5.207e^{(-03)}(P_{mean} \text{ January}) + 6.837e^{(-02)}(T_{mean} \text{ May}) + 1.313 e^{(-01)}(Corine_{133}) - 3.235 e^{(-02)}(Corine_{212}) + 3.971e^{(03)}(Corine_{312}) - 6.307e^{(-02)}(Corine_{323}))}$$

According to the results obtained, areas with highest potential risk predicted by the statistical model are in the lower altitude areas of Murcia Region (Segura Valley and Lower Guadalentín Valley), being of greater importance the areas located near forest transition environments, and far from zones of sparse vegetation (Figure 4).

DISCUSSION

In the current context of environmental changes and increasing contact at the wildlife-domestic-human interface, studies such as the present one are recommended (Villar et al., 2020), especially in relation to ubiquitous vectors such as fleas, whose ability to parasitize a wide range of hosts makes them a perfect vehicle of pathogens between wildlife and the domestic-human environment (Poulin et al., 2006).

We have found a species richness similar to that reported in other studies in the same geographical area, with the exception of *N. fasciatus*, which has not been cited as a parasite of foxes in SE Spain (Martínez-Carrasco et al., 2007). In northern Spain and the rest of European countries, the species richness differs, where the only coincident species are *P. irritans* and *C. felis*, not being usual to find citations related to *S. cuniculi* and *N. fasciatus* in red fox (Domínguez-Peñafiel et al., 2011; Foley et al., 2017; Vichová et al., 2018). In this regard, it should be noted that, in red foxes from central and northern European countries, it is common to find *Chaetopsylla globiceps* (Taschenberg, 1880), which is cited as the specific flea of the red fox (Foley et al., 2017); however, there are no records of this flea species in the Iberian Peninsula. On the other hand, *N. fasciatus*, that is considered rodent-specific, has been cited as the most prevalent species

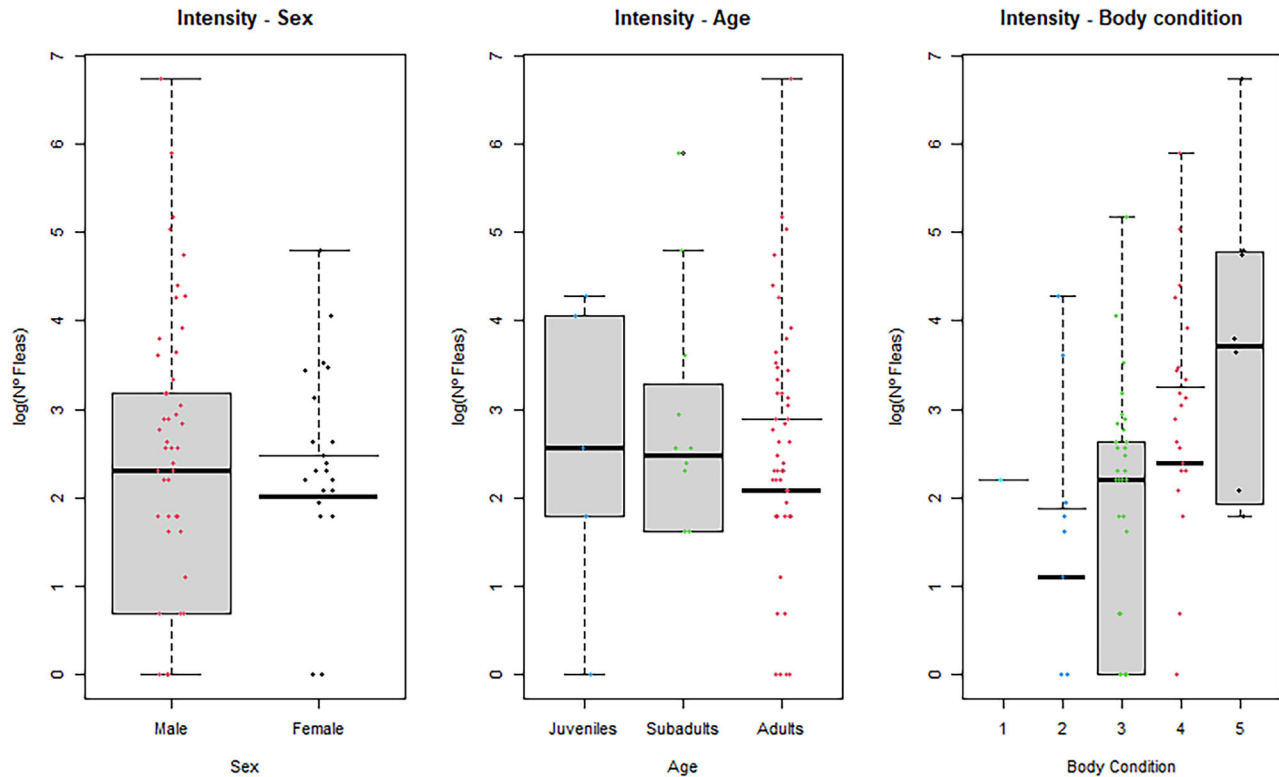


FIGURE 3 Intensity of fleas according to the sex, age, and body condition of the host. Number of fleas in log scale. Body condition (BC) categories: 1.- cachectic, 2.- thin, 3.- good BC, 4.- very good BC, and 5.- excellent BC

in small mammal populations in North-western Spain (Herrero-Cófreces et al., 2021).

Compared to previous studies in the same area (Martínez-Carrasco et al., 2007), the prevalence detected in our study was lower for *P. irritans* (75% vs. 90.1%) and higher for *C. felis* (35.2% vs. 9.1%) and *S. cuniculi* (19.3% vs. 3.6%). Additionally, overall prevalence was higher than in other European countries mainly for *P. irritans* (75%): 27.5% in Romania, 51.3% in France, 43% in Hungary, and 1.6% in United Kingdom (Foley et al., 2017).

Pulex irritans, *C. felis*, and *S. cuniculi* are fleas that belong to the family Pulicidae, all of which are cosmopolitan species with a wide range of potential hosts, including carnivores, ungulates, and bats. On the other hand, *N. fasciatus* is a member of the family Ceratophyllidae, with a predominantly Holarctic distribution, and whose main hosts are rodents and, occasionally, mustelids and viverrids. Although all four species have a wide host range, they are traditionally referred to as the human flea, cat flea, rabbit flea, and northern rat flea because these mammal species are their usual hosts, respectively, and all of them are of great epidemiological importance as disease vectors (Bitam et al., 2010). When comparing our results with those obtained in previous studies carried out in the Iberian Peninsula and other European areas, we found variability in the species richness and prevalence of flea species depending on the study area, which is probably conditioned by environmental and ecological differences (Gálvez et al., 2017). The predominance of *P. irritans* and *C. felis* could be attributed to the contact of foxes with mammals living in anthropized

areas, mainly domestic animals such as dogs, cats, and livestock. This is evidence that the use of anthropized areas by foxes carries the risk of acquiring parasites from domestic animals and synanthropic species. On the other hand, species such as *S. cuniculi* and *N. fasciatus* could indicate trophic interactions between hosts, and in this case with the fox as a predator of rabbits and rodents. In this sense, latitudinal patterns in fox diet have been described in the Iberian Peninsula, with greater consumption of lagomorphs and invertebrates in southern areas, and a higher intake of small mammals (mainly rodents) and fruits/seeds in northern regions (Díaz-Ruiz et al., 2013).

The predictive model established a certain pattern to determine the areas with the highest risk of acquiring fleas. In particular, the positive correlation of ET_0 in winter indicates that there is a greater risk of foxes having higher flea intensities, with the opposite effect occurring for ET_0 in summer. On the other hand, the positive correlations for T_{mean} in summer and P_{mean} in winter and summer would indicate an increase in intensity when these values are high. Considering that high ET_0 is related to high temperatures and reduced environmental humidity in arid and semi-arid environments, and that the Mediterranean climate is characterized by a dry summer period and wet winters, it makes sense to think of ET_0 in summer as a limiting factor due to the low availability of water in that period. In this regard, experimental studies have shown that pupal survival is greater at higher humidity, independent of temperature, and that flea larvae do not survive below 40% of environmental humidity (Krasnov et al., 2001) supporting the results obtained in the present study. Similar results have been found for *C. felis* where

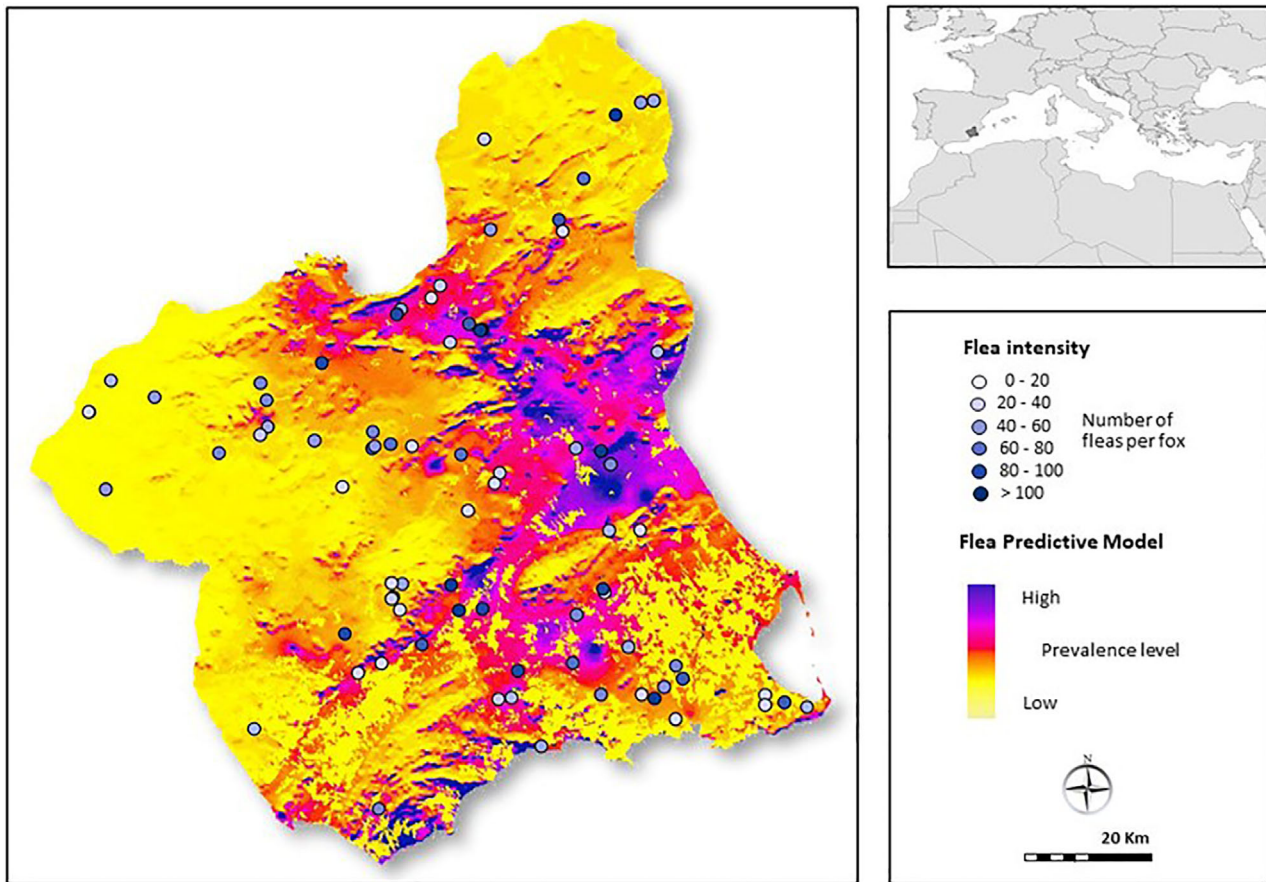


FIGURE 4 Predictive map indicating the geographic distribution of areas with the highest potential risk of flea presence in Murcia Region (SE Spain). Each point corresponds to the origin of the foxes studied; the intense blue indicates the foxes with the highest parasite intensity

abundance peaks were observed in early summer and late autumn, and for *P. irritans* with the maximum abundance peak observed in autumn, in both cases outside the summer season (Gálvez et al., 2017).

Distribution patterns of fleas in the fox population were also determined by the type of land cover and land use. Specifically, a positive correlation with forested areas (Corine₃₁₂) and urbanized areas (Corine₁₃₃) was found. The model outputs indicate higher parasite intensity in fragmented and heterogeneous habitats with different ecotones, including the availability of nearby tree cover that could serve as shelter for the fox (Castro et al., 2022). On the other hand, areas with a higher degree of urbanization, despite being more anthropized, act as transition zones to natural environments, becoming attractive areas for foxes possibly due to the greater availability of trophic resources (Gloor et al., 2001; Díaz-Ruiz et al., 2013). This result suggests that, in semi-arid Mediterranean areas, the wildlife–domestic–human interface should be given special consideration by health authorities, since the increased risk of acquiring fleas may lead to higher rates of vector-borne disease transmission in both domestic carnivores and humans (Poo-Muñoz et al., 2016). By contrast, the negative correlation for sclerophyllous vegetation (Corine₃₂₃) and permanently irrigated areas (Corine₂₁₂) shows an interaction between sclerophyllous vegetation and drier environments, with low humidity and smaller species of vegetation, making these areas not optimal for the development of the flea

life cycle (Bussotti et al., 2014). As for permanently irrigated areas, they often coincide with irrigated crops. In this areas, the homogenization of land covers results in a loss of natural habitats and the degradation of the mosaic landscape, which is possibly a deterrent for foxes, reducing their abundance in these areas and, consequently, the number of fleas on the host population (Cancio et al., 2017; Herrero-Cófreces et al., 2021; Krasnov et al., 2022).

Lastly, it is relevant to indicate the utility of the variables obtained from remote sensing techniques. In our study, the thermal spectral band of the TIRS sensor and the BSI spectral index, which show the distribution of areas of sclerophyllous vegetation, are statistically significant. Indirectly, it can also be useful to determine land surface coverages at different wavelengths thus providing useful information in study areas where thematic mapping would be difficult to obtain (Rinaldi et al., 2006).

Based on the results obtained, potential risk areas in Murcia Region are located mainly in ecotone zones between forest habitat and anthropized areas (Segura Valley and lower Guadalentín Valley). These results are consistent with the initial hypothesis, due to the preference of foxes to inhabit areas close to forest-agricultural transition zones and urban areas (Recio et al., 2015), and because of their capability to host a wide range of parasites (Foley et al., 2017; Karamon et al., 2018).

The present work emphasizes the need to carry out studies at meso- and micro-environmental scales to understand the

epidemiology of fleas, generating predictive models that allow the delimitation of potential risk areas for vector-borne disease transmission. The high prevalence of fleas found in Murcia Region compared to the rest of the Iberian Peninsula and other European countries suggests that semi-arid Mediterranean areas present environmental characteristics that allow the existence of potential flea hotspots. Evapotranspiration, summer temperature, and precipitation are the environmental factors that best predict flea distribution patterns in these areas, possibly related to the optimal conditions required by fleas to develop their extra-host life cycle. The ecotone zones between forest habitat and anthropized areas located at low altitudes are those with the highest potential risk of fox flea infestations, leading to a higher risk of vector-borne disease spread at the wildlife-domestic-human interface. In this sense, the predictive model and the risk map obtained in this study can be valuable tools to establish strategies for the prevention and control of fleas and diseases transmitted by them.

AUTHOR CONTRIBUTIONS

Roberto Pérez-Allende (RPA), Pedro Pérez-Cutillas (PPC), and Carlos Martínez-Carrasco (CMC) *conceived and designed the study*. RPA, Moisés González, Clara Muñoz-Hernández, Irene Arcenillas, Rocío Ruiz de Ybáñez, Fernando Escribano, and CMC *performed sample collection and data acquisition*. RPA, PPC, and CMC *performed data analysis, interpreted the results, and wrote the first draft of the manuscript*. All authors *critically reviewed and approved the final manuscript*.

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CONFLICT OF INTEREST

The authors declare that there are no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All procedures involving animals were approved by the Ethical Committee of the University of Murcia in compliance with laws RD32/2007 and RD1201/2005 relating to animal experimentation in Spain.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1 Supporting Information

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