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Pearsonema plica in red foxes (*Vulpes vulpes*) from semi-arid areas of the Iberian Peninsula

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ABSTRACT

The nematode *Pearsonema plica* is a parasite infecting the urinary bladder of carnivores, with a described prevalence ranging from 1 to 90%. This parasite needs earthworms as intermediate host to complete its life cycle, being the red fox (*Vulpes vulpes*) a definitive host. The objective of this study was to analyse the prevalence and intensity of *P. plica* in the red fox population from the Region of Murcia (SE Spain), an area with semi-arid Mediterranean climate. The urinary bladder, kidneys and ureters of 167 red foxes were collected at necropsy, opened and observed to detect adult parasites. The influence of host variables (sex, age and body condition using Kidney Fat Index) and environmental variables (Normalized Difference Vegetation Index, Normalized Difference Moisture Index, Bare Soil Index, temperature, radiation, evapotranspiration, precipitation, Corine Land Cover categories and distance to urban areas) were evaluated using a Generalised Linear Model. Moran index was used to evaluate the parasite spatial aggregation. The prevalence found was very low (2.4%; median abundance 0 nematodes per fox; median intensity 7.5 nematodes per parasitized fox), which contrast with those described in other red fox populations in Europe. Environmental variables had a significant influence on the occurrence of *P. plica*, being NDMI, mean summer precipitation, percentage of forest and agricultural areas positively associated with *P. plica* abundance. The south-eastern Iberian Peninsula has a semi-arid climate that hinders the development of the life cycle of this nematode, which justifies its occurrence in specific areas where there are the suitable environmental conditions for the presence of earthworms. However, although semi-arid Mediterranean areas do not seem to be favourable carnivores to be parasitized by *P. plica*, we cannot underestimate the risk that exists in those areas where, either naturally or by human activity, there are environmental factors that favor the presence of this nematode.

1. Introduction

Pearsonema plica, syn. *Capillaria plica* (Trichuroidea: Capillariidae, Rudolphi, 1819) is a nematode that infects the urinary bladder of canids and felids (Anderson, 2000). Adult nematodes are whitish and filiform, and they are found attached to the bladder mucosa and, more rarely, located in the ureters or renal pelvis (Basso et al., 2014). This nematode has an indirect life cycle with earthworms (*Lumbricina* spp.) as obligate intermediary host (Senior et al., 1980; Bowman, 2014). *P. plica* usually causes mild or asymptomatic infections. However, severe cases have occasionally been described in wild canids and dogs with outdoor access, causing cystitis, pollakiuria, dysuria and hematuria (Senior et al., 1980; Bédard et al., 2002; Callegari et al., 2010; Fernández-Aguilar et al., 2010; Rossi et al., 2011; Basso et al., 2014; Studzińska et al., 2015; Mariacher et al., 2016; Pelligra et al., 2020; Sioutas et al., 2021). Regarding wildlife, *P. plica* has been reported in several host species, including the red fox (*Vulpes vulpes*) (Bork-Mimm and Rinder, 2011; Alić et al., 2015; Aleksić et al., 2020), wolf (*Canis lupus*) (Segovia et al., 2001; Bagrade et al., 2009; Mariacher et al., 2015) and Fennoscandian Arctic fox (*Vulpes lagopus*) (Fernández-Aguilar et al., 2010). In addition, *Pearsonema* spp. has been described in brown bear (*Ursus arctos*) (Mariacher et al., 2018) and mustelids (Torres et al., 2001; Ribas et al., 2004; Petersen et al., 2018; Panayotova-Pancheva and Dakova, 2021), although the specific species has not been confirmed by molecular

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techniques. In particular, most of the studies carried out in Europe indicate that red fox (*Vulpes vulpes*) tend to have high *P. plica* prevalences, frequently exceeding 50% (see Table 1). As regards Spain, *P. plica* has been reported in foxes by Gortázar et al. (1998), Segovia et al. (2004) and Sanchis-Monsonís (2016) with prevalences of 27.3%, 40.3% and 4.2%, respectively. In this sense, it is considered that the fox may act as a reservoir of *P. plica* for other domestic (Davidson et al., 2006; Magi et al., 2014; Petersen et al., 2018; Pelligra et al., 2020) and wild canids sharing the same habitats (Aguirre, 2009; Alić et al., 2015). This prominent epidemiological role attributed to the fox in the maintenance and spread of *P. plica* is due to its wide distribution and its synanthropic and opportunistic behaviour (Jahren et al., 2020). In this regard, Iberian Mediterranean areas are dominated by agricultural landscapes and highly anthropized areas (Amaya-Castaño and Palomares, 2018), which makes possible the approach of wildlife to rural and peri-urban areas. As previous studies have shown, the presence of wildlife in anthropogenic areas increases the likelihood of contact with domestic animals and humans and, consequently, the risk of disease transmission (Deplazes et al., 2004; Morgan et al., 2008; Plumer et al., 2014; Mackenstedt et al., 2015). The semi-arid Mediterranean areas of southeastern Spain are among the zones of greatest interest for the study of epidemiological interactions at the wildlife-domestic-human interface, because they are very vulnerable to climate change, which will lead to an increase in water stress and, consequently, expected changes in the distribution of wildlife (Vicente-Serrano, 2007; Bangash et al., 2013; Andrade et al., 2021). Therefore, although the environmental characteristics of these semi-arid Mediterranean areas are not the most suitable for *P. plica* to complete its life cycle, we need to know if there are specific areas where, based on a possible increase in contact rates between foxes and dogs, there could be a risk of occurrence of this parasite.

The aim of this study was to evaluate the occurrence of *P. plica* in red foxes from the Region of Murcia (SE Spain), and to assess the influence of host and environmental characteristics on parasite abundance.

2. Material and methods

The urinary bladder, kidneys and ureters of 167 red foxes were collected between 2015 and 2021. Animals were shot in authorized hunts or roadkill in the Region of Murcia (SE Spain). The sample included 71 females and 96 males, including 51 juveniles and 116 adults. The age category was established following Harris (1978). The body condition was calculated using the Kidney Fat Index (KFI), as recommended by Winstanley et al. (1998), and based on the following formula proposed by Riney (1955): (FW/KW)x100, in which FW is the combined weight of the perirenal fat and KW the combined weight of the kidneys without fat. Urinary bladders were individually refrigerated in plastic labelled bags, as well as the kidneys and ureters of each animal, and all samples were frozen at −20 °C until the analysis.

2.1. Laboratory procedures

Urinary bladder, ureters and the renal pelvis of both kidneys were opened and inspected individually to determine the presence of nematodes. Bladder content was carefully washed and filtered through a 100 μm diameter sieve, dropped in a Petri dish and examined under a stereomicroscope, in order to identify and collect nematodes. In the case of the renal pelvis and ureters, they were directly examined using a stereomicroscope. All the parasites found were washed in distilled water and preserved in 70% ethanol until their morphometric identification according to Skrjabin et al. (1970), Butterworth and Beverley-Burton (1980) and Anderson et al. (2009).

2.2. Statistical analysis

The prevalence (P) with 95% CI, median abundance (MA) and median intensity (MI) were determined according to Bush et al. (1997). The host and environmental factors influencing parasitic abundance were evaluated by mean of a Generalised Linear Model (GLM), with Poisson distribution family. Attending to the home range of the fox (Deak et al., 2020), environmental variables were calculated for a 1 km buffer (400 ha) from the geographic location of origin of each individual using QGIS (3.16.11) software (QGIS Development Team, 2021). The environmental variables were grouped into three categories: climatic variables, spectral index and land uses (Table 2). Climate variables were obtained from annual and monthly average data series of precipitation, temperature, radiation and evapotranspiration (Ninyerola et al., 2005). Evapotranspiration was calculated from monthly and annual average values of temperature and radiation, according to Hargreaves and Samani (1985). The second dataset were obtained from the reflectance values of the land surface from the OLI and TIRS sensors of the Landsat 8 satellite (https://earthexplorer.usgs.gov/). Winter and summer seasons were taken into account for the acquisition of the images (05/08/2019 and 12/01/2020, respectively). The Normalized Vegetation Index (NDVI) related with plants photosynthesis, is calculated from 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). Attending to water stress of vegetation we used Normalized Moisture Index (NDMI) which is determined from the wavelengths of the near infrared, and the

Table 1
Prevalence (P) of *Pearsonema plica* described in foxes from Europe.

Country	Number of foxes	P (%)	Parasite detection method (*)	Reference
Sweeden	387	58.9	AN	Wolff and Bucklar (1995)
Spain	161	27.3		Gortázar et al. (1998)
Croatia	85	3		Rajkoic-Janje et al. (2002)
Hungary	100	52		Sréter et al. (2003)
Spain	399	40.3		Segovia et al. (2004)
Portugal	62	1.6		Eira et al. (2006)
Denmark	748	80.5		Saeed et al. (2006)
Norway	154	53	AN and BM	Davidson et al. (2006)
Germany	116	78	AN and E	Bork-Mimm and Rinder (2011)
Bulgary	113	56.7	AN	Kirkova et al. (2011)
Lithuania	104	93.3		Bružinskaitė-Schmidhalter et al. (2012)
Bosnia-Herzegovina	112	58	AN and E	Alić et al. (2015)
Italy	165	56.8		Magi et al. (2014)
Spain	286	4.2	AN	Sanchis-Monsonís (2016)
Denmark	247	73.7	AN and E	Petersen et al. (2018)
Italy	42	90.5		Pelligra et al. (2020)
Serbia	17	70.6		Aleksić et al. (2020)
Italy	28	75		Eleni et al. (2021)

(*) (AN): adult nematodes of *P. plica* detected by the necropsy of the bladder; (E): Eggs of *P. plica* by the urine sediment exam; (BM): adult nematodes of *P. plica* detected by bladder mucosa scraping.

Table 2
Host characteristics and environmental variables analysed in the study area (Region of Murcia, SE Spain).

		Description	Source
Host variables	Sex	Male and female levels	–
	Age	Juvenile and adult levels	Harris (1978)
	KFI (body condition)	Indicator of physiological and nutrition status of the host.	Riney (1955)
Climatic variables	Evapotranspiration	Daily potential evapotranspiration (mm/day)	Ninyerola et al. (2005)
	Radiation	Extraterrestial solar radiation (mm/day)	Ninyerola et al. (2005)
	Precipitation	Average of precipitation during the summer and winter seasons (mm)	Ninyerola et al. (2005)
	Temperate	Average of temperate during the summer and winter seasons (°C)	Ninyerola et al. (2005)
Spectral index	NDVI	Vegetation quantification calculating the difference between near-infrared (vegetation reflects) and red light (vegetation absorbs).	Elaborated by the authors based on Landsat images from https://earthexplorer.usgs.gov/
	NDMI	Combine near-infrared and short-wave infrared to measure the water content of the vegetation.	Elaborated by the authors based on Landsat images from https://earthexplorer.usgs.gov/
	BSI	Indicator of soil variations combining short-wave spectral bands and blue, red, near-infrared.	Elaborated by the authors based on Landsat images from https://earthexplorer.usgs.gov/
Land cover	CLC category 1	Artificial surfaces	CORINE Land Cover
	CLC category 2	Agricultural areas	CORINE Land Cover
	CLC category 3	Forest and seminatural areas	CORINE Land Cover
	CLC category 4	Wetlands	CORINE Land Cover
	CLC category 5	Water bodies	CORINE Land Cover
	Urbanization distance	The distance of the fox location point from an urban settlement.	Elaborated by the authors based on CORINE Land Cover (CLC2018)

Short-Wave Infrared ρ_{SWIR1} (1.57–1.65 μm). Finally, Bare Soil Index (BSI), reports the difference in spectral behaviour between bare soil and sparsely vegetated areas. For that, 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. Moreover, the thermal infrared band ρ_{TIR1} (10.60–11.19 μm) which estimates soil moisture and thermal mapping is applied from the TIRS sensor. Land cover composes the third dataset of environmental variables, where the main source of data collection was CORINE Land Cover (https://land.copernicus.eu/pan-european/corine-land-cover/clc2018).

Variance Inflations Factors (VIF) was performed to evaluate variable with high collinearity, and reduce the number of factors in the model. VIF values above 5 were discarded, as they were considered with high collinearity. Akaike's Information Criterion (AIC) (Akaike, 1974) was used in order to select the best model. R software 4.1.2 (R Core Team, 2021) software was used and significance threshold was established for p value < 0.05. Package "usdm" was used to calculate VIF values (Naimi et al., 2014).

In order to measure the spatial autocorrelation based on the locations and values of the entities simultaneously, the Moran index was applied. As a result, it provides a z-score and p-value, which indicate statistical significance. A positive value of Moran index indicates a tendency towards clustering, while a negative value indicates a tendency towards dispersion; values near 0 denote a random distribution.

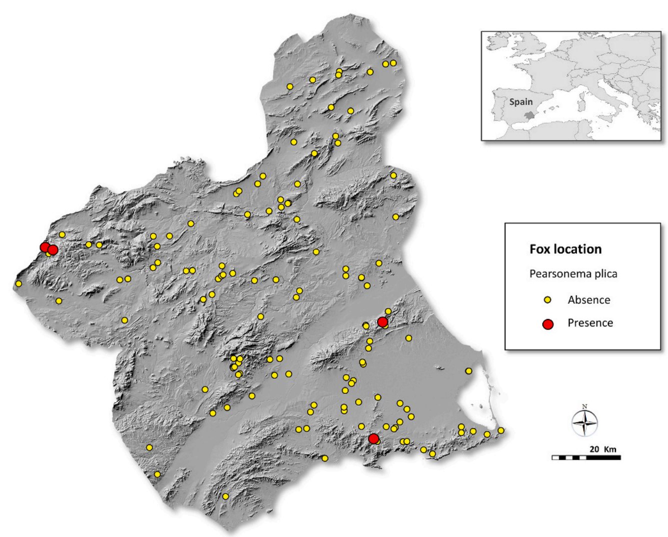

Fig. 1. Locations of the foxes studied in the Region of Murcia (SE Spain). *Pearsonema plica* negative (yellow dots) and positive (red dots) foxes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Results

Pearsonema plica was detected in four foxes (prevalence of 2.4%; 0.07–4.68, 95%CI; Fig. 1), all of them adult females (MA 0; MI 7.5, range 2–18). A total of 35 nematodes were collected, all of them found in the host bladder, and none in the ureters or renal pelvis. No macroscopic alterations compatible with cystitis were observed in the parasitized foxes.

The morphometric characteristics of the isolated adult nematodes coincided with those previously described for *P. plica* for Skrjabin et al. (1970), Aleksić et al. (2020), Pelligra et al. (2020) and Eleni et al. (2021). Among the most important characteristics, are including the vulva appendage and the colourless, lemon-shaped eggs in females, as well as the characteristic triangular shape of the terminal caudal ala in males with a thin and long spicule, some of them shown in Fig. 2.All four positive foxes were adult females, although neither sex, age nor KFI were statistically significant when GLMs were performed. Winter values of Normalized Difference Moisture Index (NDMI), mean summer precipitation, distance to urban areas and three CORINE Land Cover (CLC) categories (anthropic surfaces, agricultural land and forestry areas) were the variables included in the best model (AIC 148.14; explained variance 58%) (Table 3). These explanatory variables were positively related to

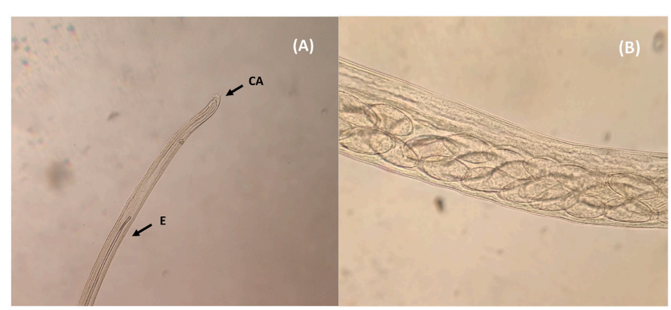

Fig. 2. (A) Posterior end of *P. plica* male where triangular caudal ala (CA) and spicule (E) can be observed. (B) Eggs contained in a *P. plica* female.

Table 3
Environmental and host variables that predict *Pearsonema plica* occurrence in red foxes from Region of Murcia (SE Spain).

Coefficients	Estimate	Std. Error	P-value
Intercept	−4.076e+02	1.843e+02	0.027023
NDMI of winter	1.620e+01	4.533e+00	0.000353
Urbanization distance	1.869e-04	6.102e-05	0.002186
Artificial surface	1.162e-01	5.301e-02	0.028401
Agricultural land	1.116e-01	5.280e-02	0.034573
Forestry areas	1.118e-01	5.285e-02	0.034370
Mean summer precipitation	3.533e-02	7.012e-03	4.67e-07

parasite abundance. From a spatial point of view, the infection was randomly distributed (Moran index = 0,017; z-score = 0.281; p-value = 0,778), meaning that it does not follow a specific spatial pattern.

4. Discussion

The present study analysed the spatial distribution and epidemiological characteristics of the bladder nematode *P. plica* in the Region of Murcia, a semi-arid Mediterranean environment of the Iberian Peninsula. The prevalence and number of this parasite species in red foxes were very low (2.4%, 35 specimens) as expected in a dry area. This very low occurrence contrast with high *P. plica* prevalences that have been reported in foxes from other studies carried out in Europe (see Table 1). In our study, no significant differences were found in relation to host variables (sex, age and KFI), may be due to the small number of positive foxes detected. In this regard, other authors also reported no differences of parasitized foxes by *P. plica* (Davidson et al., 2006; Eira et al., 2006; Alić et al., 2015; Pelligra et al., 2020), or even these variables were not analysed at all (Aleksić et al., 2020; Eleni et al., 2021). In contrast, Bork-Mimm and Rinder (2011) found higher prevalence in male foxes, although differences were not significant. On the other hand, Petersen et al. (2018) indicated that *P. plica* probably has an accumulative effect in bladder of foxes, and hence adults would report the most positive cases.

It should be noted that at least two *Pearsonema* species have been confirmed so far: *P. plica* and *Pearsonema feliscati* in felids (Bowman et al., 2002; Pelligra et al., 2020). It remains to be confirmed by molecular techniques whether the specimens found in mustelids and ursids correspond to either of these two species or whether, on the contrary, they are different *Pearsonema* species. In this regard, some studies suggest the possibility of the existence of paratenic hosts that participate in the life cycle of *Pearsonema* spp. and, consequently, could be contributing to the maintenance and dispersion of this parasite in the ecosystem (Senior et al., 1980; Bowman et al., 2002; Rossi et al., 2011). This hypothesis is based on the fact that certain host species parasitized by *Pearsonema* spp. have prevalences that apparently do not match with their diet (Seville and Addison, 1995; Petersen et al., 2018). Specifically, fox's diet is not preferentially based on earthworms' consumption, in contrast to other carnivore species, such as badgers (*Meles meles*) or raccoon dogs (*Nyctereutes procyonoides*), in which this intermediate host constitutes an important part of their diet; however, the prevalence described in these two carnivore species is much lower than that detected in foxes from Europe (Torres et al., 2001; Petersen et al., 2018). Thus, further research is needed to understand more precisely what biological factors are involved in the transmission of *Pearsonema* spp. in wild carnivores. The prevalence of *P. plica* found in this study closely follows the result reported in foxes by Rajković-Janje et al. (2002) in Croatia, with a prevalence of 3%, and other studies carried out in the Iberian Peninsula, as Eira et al. (2006) in Dunas de Mira (Portugal) with a prevalence of 1.6%, or Sanchis-Monsonís (2016), that detected a prevalence of 4.2% in Valencian Community (Spain). These results contrast with those found in other studies carried out in mountainous areas of the Iberian Peninsula, where higher prevalences have been described: Andorra (60.4%), the Cantabrian Mountains (36%) and Montseny (35.5%) (Gortázar et al., 1998; Segovia et al., 2004); these locations present a higher environmental humidity than our study area, with greater amount of precipitation and, consequently, with the environmental characteristics that favor the presence of earthworms (Segovia et al., 2004).

The results obtained in this research revealed the importance of environmental factors as NDMI of winter and mean summer precipitation values for parasite occurrence. Both variables are associated to humidity in the environment and, so, related with areas where the intermediate hosts are frequent (Macdonald, 1980). The positive association with forests can be explained by larger shadow surface, which leads to a higher rate of soil moisture, providing also optimal conditions for the presence of earthworms (Sankar and Patnaik, 2018; Singh et al., 2019). Likewise, agricultural lands where *P. plica* has been detected in Murcia usually are irrigated areas associated with higher water and humidity availability. Our findings agree with those of Gortázar et al. (1998), who described that *P. plica* prevalence was associated to the presence of agricultural lands, being the prevalence of this parasite higher in foxes from irrigated areas (31.7%) compared to those from dry areas (23%).

The low prevalence reported by Eira et al. (2006) and Sanchis-Monsonís (2016) were referred to areas with scarce precipitation and high temperatures, which are unfavourable conditions for the development and availability of earthworms. Our study area present marked aridity (<400 mm annual precipitation), summer drought, scarce and stormy precipitations, and high evapotranspiration rates (Gil-Guirado and Pérez-Morales, 2019).

Many of the studies that have investigated the presence of *P. plica* included, apart from the examination of the urinary bladder, the analysis of the urine sediment (Table 1). This method allows the detection of *P. plica* eggs and so, the infection even though no adult nematodes have been found in the bladder (Aleksić et al., 2020). In this sense, Alić et al. (2015) found no statistically significant differences when comparing prevalence results between the urine and bladder mucosa analyses, suggesting that, at least after the death of the individual (as in our study), the examination of the bladder, without performing the analysis of the urine sediment, provides a confident diagnosis. In our research, bladder mucosa, ureters and renal pelvis were examined using a stereomicroscope, which minimize the risk of false negative results, since even the detection of immature nematodes is possible by careful exam of the studied samples using a stereomicroscope.

Our survey confirms the low prevalence of *P. plica* in semi-arid Mediterranean areas, mainly because the environmental conditions in the southeast of the Iberian Peninsula are not suitable for the presence of earthworm, so the life cycle of the parasite cannot be completed. However, despite this prevalence, it should be highlighted that the epidemiological risk for domestic animals exists, since in these dry areas, water and trophic resources are often more abundant where there is greater human activity, like agricultural lands, making them attractive habitats for foxes. Therefore, in these anthropized areas it is likely the presence of all key species for the development of *P. plica*: earthworms, foxes as natural reservoirs of the parasite, and pets with free access to these shared areas. We consider that further research on the sylvatic cycle of this parasite is advisable, aiming to (1) determine whether there are other intermediate or paratenic hosts that could be involved in the maintenance of the *P. plica* life cycle, and (2) if humid microclimate areas in the semi-arid Mediterranean bioclimatic region are hotspots for this nematode of wild and domestic carnivore species.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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