First epidemiological data on Spirocerca vulpis in the red fox: A parasite of clustered geographical distribution

Published version:

DOI:10.1016/j.vprsr.2019.100338

(Article begins on next page)

Gloria Sanchis-Monsonís[1], Angela Fanelli[1], Paolo Tizzani, Carlos Martínez-Carrasco, First epidemiological data on *Spirocerca vulpis* in the red fox: A parasite of clustered geographical distribution, Veterinary Parasitology: Regional Studies and Reports, Volume 18,2019,100338, ISSN 2405-9390,
https://doi.org/10.1016/j.vprsr.2019.100338

1 These authors contributed equally to this work

First epidemiological data on *Spirocerca vulpis* in the red fox: a parasite of clustered geographical distribution

Gloria Sanchis-Monsonís[a,☐], Angela Fanelli[b,☐], Paolo Tizzani[c*], Carlos Martínez-Carrasco[a]

(a) Department of Animal Health, Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, Murcia, Spain.

(b) Postgraduate Specialization School in "Infectious Disease of Animals". Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy.

Angela Fanelli: angelafanelli6894@gmail.com;

(c) Department of Veterinary Sciences, University of Turin, Grugliasco (Turin), Italy.

Paolo Tizzani: paolo.tizzani@unito.it

☐ These authors contributed equally to this work

* Corresponding author: paolo.tizzani@unito.it (Paolo Tizzani) - Department of Veterinary Sciences, University of Turin, Largo Paolo Braccini 2, 10090 - Grugliasco (Turin), Italy.

Highlights

- Epidemiology of *Spirocerca vulpis* is described for the first time
- No statistical difference was found in parasite intensity between males and females
- Climatic variables influence the distribution of the parasite
- In foxes, *S. vulpis* shows a clustered geographical distribution

Abstract

This is the first study describing the epidemiology of *Spirocerca vulpis* after its description as a new species in 2018. During the period 2006-2013, a total of 286 red foxes (*Vulpes vulpes*) from the Valencian Community (southeast Spain) were necropsied. Based on data collected, *S. vulpis* prevalence and intensity were calculated, as well as the spatial distribution of this nematode. Influence of host (sex and age) and environmental factors on *S. vulpis* occurrence was evaluated. MAXENT software was used to model and predict the parasite distribution. Continuous and discrete prediction maps were built using ArcMap 10.6. The prevalence of *S. vulpis* was 22% (63/286; 95% CI: 17.4-27.3), and the median intensity was 5 (IQR 11) nematode specimens. No significant difference in term of intensity was found between males and females; regarding the host age, *S. vulpis* was found only in adult foxes, with the exception of one juvenile individual. The distribution of *S. vulpis* in foxes was skewed to the left, highlighting that parasite infection affects few individuals within a population, with parasitized animals being responsible to maintain the infection at the population level. The majority of parasitized foxes had a parasite burden lower than eight parasites/individual. *S. vulpis* distribution in Valencian Community presents sharply defined areas in which there are optimal environmental conditions for maintaining the life cycle of this parasite. Climatic variables and altitude are the main factors influencing the parasite presence. Our results indicate that *S. vulpis* has epidemiological characteristics similar to those of *S. lupi* and, therefore, based on the phylogenetic proximity of both nematode species, it is likely that coprophagous beetle species might play a key epidemiological role in the maintenance of this newly described *Spirocerca* species. Moreover, it is currently unknown if *S. vulpis* can infect the dog and other wild canid species apart from the red fox and, if so, what are the pathogenic effects on these host species. Therefore, it is necessary to continue investigating the epidemiology of this parasite in order to know the range of appropriate host species. This information will enable to know if *S. vulpis* endemic areas should

be considered as health risk points for dogs, especially for the most exposed, such as those living in rural areas, and hunting dogs.

Keywords: Geographical distribution; red fox; *Spirocerca vulpis*; southeast Spain

1. INTRODUCTION

Until recently, spirocercosis in domestic and wild canids was believed to be caused exclusively by *Spirocerca lupi*, a Spirocercid nematode found worldwide, especially in tropical and subtropical regions (Van der Merwe et al., 2008; Rothmann and de Vaal, 2017). Spirocercosis is a disease that can become fatal in dogs and wild canids (Joubert et al., 2005; Rinas et al., 2009; Morandi et al, 2014), so it is a concern for veterinarians in countries where the disease has been detected (Anataraman and Krishna, 1966; Dixon and McCue, 1967; Brodey et al., 1977; Ramachandran et al., 1984; Lobetti, 2000; Mylonakis et al., 2001; Reche-Emonot et al., 2001; Mazaki-Tovi et al., 2002; Oliveira-Sequeira et al., 2002; Le Sueur et al., 2010).

A new species, *Spirocerca vulpis*, was recently described in red foxes (*Vulpes vulpes*) from Europe, based on morphometric analyses and molecular identification (Rojas et al., 2018a, b). So far, the presence of *S. lupi* in foxes from different European countries has been cited (Gortázar et al., 1998; Segovia et al. 2001; Shimalov and Shimalov, 2003: Segovia et al. 2004; Eira et al., 2006; Ferrantelli et al. 2010; Diakou et al. 2012; Morandi et al. 2014; Magi et al. 2015; Valcárcel et al., 2018). However, as suggested by Rojas et al. (2018a, b), these studies were not based on a detailed morphological and genotypic analysis of the specimens found, so these parasites may have been misclassified as *S. lupi*. In the light of these findings, previous studies of spirocercosis in foxes need to be re-evaluated. For example, an interesting aspect of vulpine spirocercosis is that parasite nodules are usually located in the gastric wall and the omentum (Prokopic, 1960; Segovia et al., 2001; Ferrantelli et al., 2010; Diakou et al., 2012; Al-Sabi et al., 2014; Rojas et al. 2018b; Valcárcel et al., 2018) and not in the esophagus, where *S. lupi* nodules are most frequently located (Bailey, 1963, 1972; Anderson, 2000; Van der Merwe et al., 2008).

The life cycle of parasites in wildlife is conditioned, among other factors, by environmental characteristics, especially in those parasites transmitted by the predation of intermediate or paratenic hosts (Poulin and Morand, 2000; Bozick and Real, 2015). So far, very few studies have analyzed the epidemiological characteristics of spirocercosis in wild canids. On the other hand, the description of the new species *S. vulpis* makes it necessary to investigate what these characteristics are, in order to better understand their geographical distribution and the environmental factors that may condition their maintenance and dispersion (Huang et al., 2014). In this way, we will be getting progressively more information that will allow us to know the epidemiological risks that occur and, consequently, appropriate prevention measures that can be implemented. In this context, carrying out basic epidemiological investigation on one side, and developing predictive habitat distribution models may provide important information to fill these gaps of knowledge. Several species distribution models (SDMs) are currently used to predict species distribution; among them, Maximum Entropy (MAXENT) has become increasingly popular in recent years. As other SDMs, MAXENT algorithm relates the locations of species with the environmental characteristics, in order to estimate the response function and contribution of each factor, as well as to predict the probability of species presence (Fourcade et al., 2014).

The red fox is a generalist predator with a wide distribution and a high ecological plasticity (Dell'Arte et al., 2007), being able to feed on ample trophic resources, as small prey, carrion and garbage. This wild canid is present in a wide range of habitats in the Iberian Peninsula (Ballesteros, 1998; Gortázar, 2007; Jiménez et al., 2012), with densities of 0.7-2.5 foxes/Km^2, depending on environmental conditions (Gortázar et al., 1998; Sarmento et al., 2009).

In the aforementioned study of Rojas et al. (2018b), specimens of nematodes obtained from foxes of the Valencian Community (southeastern Spain) were analyzed and anatomopathological description of lesion provided, confirming that they belong to the species *S. vulpis*. Therefore, the study of the fox population of this area offers a valuable opportunity to obtain, for the first time, epidemiological data that will be very useful to understand which factors influence the presence of this parasite in Mediterranean habitats of the Iberian Peninsula.

Considering the above, the objectives of this study were (i) to describe the prevalence, abundance, intensity and parasite aggregation of *S. vulpis* in the red fox population of the Valencian Community, (ii) to evaluate the environmental variables influencing the distribution of *S. vulpis*, and (iii) to identify and locate on a map, the areas in the Valencian Community with significant higher risk of spirocercosis occurrence .

2. MATERIAL AND METHODS

2.1. Study area and animals sampled

During 2006-2013, 286 foxes (151 males and 135 females; 225 adults and 61 juveniles) from the Valencian Community (SE Spain – Figure 1) were necropsied in the context of an official wildlife surveillance program. Foxes were hunted under official permits or killed by traffic accidents. The climate of the study area is typically Mediterranean, with hot, dry summers and mild winters; average temperature range in the area is 11-18 degrees, and average precipitation is 400-600 mm (Piqueras, 1999; Aguilella et al., 2009). The estimation of age was done by tooth replacement and wear (Saenz de Buruaga et al., 1991), classifying foxes into two categories: juvenile (under six month-age) and adult (the rest).

During the necropsy, all nodules suspected of being caused by *Spirocerca* spp. were opened and the nematodes washed and preserved in 70% ethanol. A total of 26 randomly selected nematodes were analyzed by molecular and morphometric techniques (for more details, see Rojas et al., 2018b), confirming that they were specimens of *S. vulpis*. Subsequently, the remaining isolated nematodes were identified as *S. vulpis* based on the morphometric characteristics proposed by Rojas et al. (2018b).

2.2. Epidemiological descriptors

The distribution of the parasite was evaluated by mean of the epidemiological indexes of prevalence (percentage of infected animals), abundance (number parasites/total animals) and intensity (number parasites /positive animals), according to Bush et al. (1997). As indices of aggregation, we computed the variance-to-mean ratio, obtained by dividing the mean parasite abundance by its variance. Mean parasite abundance and variance were obtained considering the global parasite load for all the sampled animals. The distribution is over-dispersed if this ratio is >1, and under-dispersed (aggregated) if this value is <1 (Barbour and Pugliese, 2000; Vale et al., 2013). The shape of *S. vulpis* distribution in the sampled population was graphically evaluated by mean of a density plot (R Core Team, 2018), which uses kernel smoothing to display frequency values, allowing for smoother distributions. This plot helps to evaluate where values are concentrated. All the descriptors were stratified by sex and age category.

To evaluate the effect of host factors (sex and age category) on parasite distribution, we applied the approach suggested by Rózsa et al. (2000); concretely, prevalence values were compared using the Fisher's exact test, and frequency distribution of parasite intensity and abundance with Mann–Whitney's U-test. All statistical analysis were performed using R (R Core Team, 2018).

2.3. Species Distribution Models (SDMs)

Seventeen environmental variables were used to build the predictive model (Table 1). The monthly values of climatic and Normalized Difference Vegetation Index (NDVI) data were grouped (average value) in "dry period" (DP - July to October) and "wet period" (WP - January to June and November to December). All the rasters were rescaled at a resolution of 1 km, aligned and re-projected using the same CRS (WGS84). This process was done using ArcMap 10.6 (Environmental Systems Research Institute -ESRI-, 2017). Before building the model, the HH package was used to compute Variance inflation factors (VIFs) and evaluate collinearity among the independent variables (Heiberger, 2018). Variables with VIF >10 were excluded from the model.

As result of collinearity evaluation, only Digital Elevation Model (DEM), Latitude, Longitude, Temperature (T) min (WP), T min (DP), T average (WP), T average (DP), NDVI (WP), NDVI (DP), Precipitation (WP), and Precipitation (DP), were retained. Model was built using a backward selection approach. Rasters were entered in MAXENT (Phillips, 2017) and the software was run dividing the presence data into 80% of training points and 20% of test points. Regularization parameter was set to "3" in order to control for model overfitting (Radosavljevic and Anderson, 2014). The most parsimonious model was selected using ENMTools v1.3 (Warren et al., 2010), to compute the Akaike Information Criterion (AIC) (Akaike, 1973). Variables were progressively removed based on the jacknife test (lower contribution in the AUC score). Maximum training sensitivity plus specificity was selected as threshold to convert continuous prediction (logistic) into binary output.

2.4. Variable Importance and Model performance

Permutation importance (PI) was used to assess the contribution of each environmental factor. PI value determines the contribution of each factor by measuring how much the model decrease in quality when the variable is not selected. Response curves were also generated to interpret the relationship of the environmental factors with the probability that *S. vulpis* was present. To assess performance of the MAXENT model, area under the curve value (AUC) was computed. AUC compares the model sensitivity (true positives) against "1 – specificity" (false positives) over the entire range of threshold. This curve represents the probability that a randomly chosen presence site will be ranked as more suitable than a randomly chosen pseudo-absence site. A model that does not perform better than random will have an AUC of 0.5, whereas a model with perfect discrimination would reach a value of 1. MAXENT output provides also the "regularized training gain" parameter which describes how much better the MAXENT distribution fits the presence data compared to a uniform distribution. Exponential training gain gives the average ratio of the likelihood assigned to an observed presence location to the likelihood assigned to a background location.

3. RESULTS

The shape of *S. vulpis* distribution is presented in Figure 2. Seventy-eight percent of the animals (223 foxes) were not infected by *S. vulpis*, and the majority of the infected animals show low density of parasite/host, thus the curve results to be skewed to the left. Within the positive animals, 65% (41/63) harbored between one to eight *S. vulpis* specimens, and 35% (22/63) more than eight. Maximum parasite load (44 nematodes) was found in two adult foxes (a male and a female). The total number of nematodes detected was 605. The histological description of the S. vulpis nodules, as well as their anatomical location, can be found in Rojas et al. (2018b).

The prevalence, abundance and intensity values are reported in Table 2. The distribution of *S. vulpis* in the fox population of Valencian Community was strongly conditioned by the age of the host. Specifically, only one of the infected foxes was juvenile, being the remaining ones adults. In this sense, the Fisher's exact test was significant for age (p value <0.01) with odds ratio of 0.044 (i.e., less risk of being infected in juveniles).

Regarding the effect of sex, the Fisher's exact test on parasite prevalence (p value = 0.25) and the Mann–Whitney's U-test parasite on abundance (p value = 0.25) and intensity (p value = 0.48) were not statistically significant at alpha level of 0.05.

The predictive accuracy of *S. vulpis* model was very high (AUC = 0.91), and the training gain was 0.147. This nematode shows a sharply defined spatial pattern with the most suitable area located in the Western and Central part of the Valencian Community (Figure 3). In particular, according to the sample analysed, a geographical cluster of spirocercosis was identified in the "Reserva Valenciana de Caza Muela de Cortes" (Ayora Valley), where 25 foxes over 32 were infected (prevalence = 78.1%). The probability of occurrence is shown in the left part of the figure by the darker shade of yellow. In particular, the application of a cut-off value shows, on the right part of the figure, the area considered as suitable for parasite occurrence.

Table 3 shows estimates of relative contributions of the environmental variables to the Maxent model. Among those influencing parasite distribution, the minimum temperature of the wet period had the highest permutation importance (PI=51.5), followed by the average temperature of the dry period (PI= 33.9) and the altitude (PI= 4.2). The probability of presence of *S. vulpis* increases from -2ºC and peaks at 4ºC in wet period, while in dry period it drops above 21ºC. Regarding altitude, the model identifies optimal condition for the presence of *S. vulpis* around 300 metres a.s.l. These three variables explained almost 90% of the prediction accuracy of the model (Figure 4). The remaining part was due mainly to latitude (PI=3.6) and longitude (PI=3.6) effect.

4. DISCUSSION

This study is the first to be carried out worldwide to determine the epidemiological characteristics of *S. vulpis* in foxes. Since its recent description, there has been no published study describing spirocercosis in foxes or other species of canids, whether domestic or wild. In accordance with Rojas et al (2018b), the results of previous studies in which *S. lupi* has been described in foxes should be evaluated with caution, since no precise identification techniques or molecular methods were used in any of them. Fortunately, we now have the morphometric characteristics of *S. vulpis* that differentiate it from *S. lupi* (Rojas et al., 2018), so it is to be expected that, from now on, the number of studies confirming the presence of *S. vulpis* in foxes and, perhaps, in other canine species, will increase. Therefore, we can only compare our results with those of previous studies in which the presence of *S. lupi* in foxes has been described, assuming that it is possibly the same species that we have found in the foxes of the Valencian Community, i.e. *S. vulpis*.

In Europe, *S. lupi*-like nematodes have been described in foxes with prevalence of 2.1% in Belarus (Shimalov and Shimalov, 2003), 9.16% in Sicily, Italy (Ferrantelli et al. 2010), and 23.5% in northwestern Italy (Magi et al., 2015). In the Iberian Peninsula, the distribution of *S. lupi* in foxes is irregular with a generally low prevalence. Specifically, the prevalence in the Ebro Valley was 2.5% (Gortázar et al., 1998), 12.9% in Portugal (Eira et al., 2006) and, recently, Valcárcel et al. (2018) found a prevalence of 18% in Ciudad Real (Central Spain). Such variability in parasite prevalence can be related to the different habitat, period and environmental conditions in the different study areas investigated. However, in some studies, the prevalence was higher, ranging from 29.1% to 65.4% in central-western areas of the Iberian Peninsula (Segovia et al., 2004; González et al., 2009; Calero-Bernal et al., 2011). In our study, the prevalence of *S. vulpis* shows intermediate values (22%). But, as mentioned above, a retrospective analysis evaluating the correct identification of *S. lupi* in previous studies would be necessary to have more accurate data for epidemiological comparison.

No influence of host sex was detected on parasite prevalence, abundance or intensity, while a significant effect of age and environmental factors was identified. In particular, climatic variables had the highest influence on the *S. vulpis* distribution, with occurrence of spirocercosis limited to very specific range for temperature and precipitations. For this reason, the geographic distribution of vulpine spirocercosis is restricted to clustered areas in which the appropriate microclimatic conditions are present so that its intermediate hosts can develop and maintain the parasite life cycle, as demonstrated in other fox nematodes (Maksimov et al., 2017; Čabanová et al., 2018).

In general terms, the metazoan parasite intensity follows a negative binomial distribution when studying wildlife populations (Shaw et al., 1998; Poulin, 2007). As expected, the distribution of *S. vulpis* in red foxes is skewed to the left, highlighting that parasite infection involves few individuals within the studied population, with these parasitized hosts being responsible to maintain the infestation at the population level. The variance-to-mean ratio was lower than one, indicating that *S. vulpis* presents a aggregated distribution within the host population.

Age was found to have a significant effect on *S. vulpis* distribution; concretely, only one juvenile fox was infected by *S. vulpis.* This finding could be related to the length of the prepatent period of *Spirocerca* spp. In the case of *S. lupi* in dogs, this period is 3-8 months (Sen and Anantaraman, 1971; Mazaki-Tovi et al., 2002). Assuming a similar prepatent period for *S. vulpis*, this may explain why macroscopic nodules have not been detected in juvenile foxes. In adults in fact, the parasite has a longer period to develop macroscopic lesions detectable during the necropsy. In addition, the chance of becoming infected increases with age. This result is consistent with the study by Aroch et al. (2015), who found that *S. lupi* is significantly more prevalent in adult dogs, possibly because they are more likely to have been infected during their lifetime.

In our study, there were no significant differences between males and females, coinciding with previous studies in which host sex is not a significant risk factor for spirocercosis (Van der Merwe et al., 2008; Valcárcel et al., 2018).

Regarding the spatial distribution of the parasite, the presence of *S. vulpis* in Valencian Community is restricted to specific areas. This pattern is similar to that described by previous studies in which *S. lupi* in dogs has been shown to be endemic and restricted to well-defined areas (Bailey, 1972;

Mazaki-Tovi et al., 2002). The life cycle of *S. vulpis* is not known at present; however, we assume that it could be similar to that of *S. lupi*, in which coprophagous beetles act as intermediate hosts (Bailey et al., 1963), as well as birds, lizards and small mammals as paratenic hosts (Van der Merwe et al. 2008). Our study shows that the Ayora valley and Muela de Cortes are two endemic focuses in which the prevalence of *S. vulpis* is very high. This is probably due to the dryness of the environment and the presence of a low shrub cover, which are factors directly related to the presence of coprophagous beetles (Carvalho and Gomes, 2004). Therefore, in areas of high prevalence of *S. vulpis* there are climatic conditions that favor the presence of these intermediate hosts and, consequently, the biological cycle of the parasite can be maintained (Bailey, 1963). Regarding the significant effect of altitude on the distribution of *S. vulpis*, it might be related to a higher density of foxes at lower altitudes (Sándor et al., 2017), but more studies are needed to know in more detail what the influence of this factor is on the epidemiology of the parasite.

Concerning *S. lupi*, it has also been suggested that the incidence of infection may be up to 85% in endemic areas, and related to the degree of rural development, utilization of pesticides, disease control efforts (Van der Merwe et al., 2008). In our study, the detection of *S. vulpis* in specific areas is also highlighted by the significant role of latitude and longitude values in the models, which means that S. vulpis distributes in well-defined clusters.

5. CONCLUSIONS

This is the first study describing the epidemiology of *S. vulpis*, a new species recently found in red foxes. The importance of our results is represented by the fact that our data are the very first available epidemiological data on this parasite, including the description of basic parameters like prevalence, intensity and abundance at population level. Moreover, the environmental factors influencing parasite risk of occurrence are described. This data will be important not only to understand the epidemiology of the disease but also to provide a better evaluation of the possible risk of infection for domestic canids.

It is currently unknown whether *S. vulpis* can infect the dog and other wild canids in which *S. lupi* has been described. Possibly both nematode species have a very similar life cycle, so it is expected that the epidemiological characteristics may also be similar, although future studies are needed to elucidate this. However, we can assume that, in the hypothetical case that *S. vulpis* may affect more than one species of canine, there would be a health risk for dogs, especially those living in rural areas and hunting dogs, which are more exposed (Mylonakis et al., 2001).

The red fox is a carrier of nematodes with importance from a health point of view, either because of its zoonotic character or because they are parasites shared with other domestic or wild animals. In this context, predictive models are important tools for understanding which factors influence the parasite distribution, and thus map the risk of disease transmission to domestic dogs and other wildlife species. Although there is no census of dogs available in the study are in general, and specifically in the areas with the highest risk of spirocercosis, we can assume that shepherd and hunter dogs, as well as pets, can have an increased risk of disease transmission in this areas. However, no case of S. vulpis in dogs has yet been described, so it will be necessary to study in more detail the life cycle of the parasite in areas of high prevalence and confirm the possibility of parasite transmission to domestic animals.

The application of MAXENT algorithm provides valuable insights on the relationship between parasite presence and predictors. Climate variables are able to affect the prevalence, intensity and geographical distribution of helminths, directly influencing free-living larval stages and indirectly influencing invertebrate and vertebrate hosts. Very few studies are available on the spatial factors affecting parasite distribution, so we encourage further analysis to better understand the factor affecting parasite presence and distribution.

Our model demonstrated high predictive performance, and it has been shown in scientific literature that models for the specialist species had consistently strong performances as a consequence of the requirement for explicit environmental variables and that are easily defined by predictive models (Evangelista et al., 2008). From a practical perspective our model could be considered a useful tool for the application of preventive and control strategies to limit the diffusion of the disease and the risk of infection for domestic animals.Nevertheless, we should consider that a) data resolution might affect the explanatory power and predictive accuracy; b) variable selection may influence the quality of the model. A different set of variables may have different results and discriminatory capacity.

ACKNOWLEDGEMENTS

This study was supported by the Conselleria of Agriculture, Environment, Climate Change and Rural Development, Game and Fish Service,. The authors thank the team of veterinarians of the hunting and fishing service, Miguel Ángel Sánchez Isarria, Iris Garcia Bacete and Víctor Lizana Martín for their invaluable contribution in obtaining samples, as well as the group of environmental agents and hunters.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

6. REFERENCES

Aguilella, A., Fos, S., Laguna, E., 2009. Catálogo valenciano de especies de flora amenazadas. Colección Biodiversidad, 18. Generalitat Valenciana Conselleria de Medi Ambient, Aigua, Urbanisme i Habitatge, Valencia.

Akaike, H., 1973. Information Theory and an Extension of the Maximum Likelihood Principle. In: Petrov, B.N., Csaki, F. (Eds.), Proceedings of the 2nd International Symposium on Information Theory. Akadémiai Kiadó, Budapest, pp. 267–281.

Al-Sabi, M.N.S., Hansen, M.S., Chriél, M., Holm, E., Larsen, G., Enemark, H.L., 2014. Genetically distinct isolates of *Spirocerca* sp. from a naturally infected red fox (*Vulpes vulpes*) from Denmark. Vet. Parasitol. 205, 389–396.

Anataraman, M., Krishna, S., 1966. Experimental spirocercosis in dogs with larvae from a paratenic host, *Calotes versicolor*, the common garden lizard in Madras. J. Parasitol. 52, 911–912.

Anderson, R.C., 2000. Nematode parasites of vertebrates. Their transmission and development, 2nd ed. CABI 36 Publishing, UK.

Aroch, I., Markovics, A., Mazaki-Tovi, M., Kuzi, S., Harrus, S., Yas, E., Baneth, G., Bar-El, M., Bdolah-Abram, T., Segev, G., Lavy, E., 2015. Spirocercosis in dogs in Israel: A retrospective case-control study (2004–2009). Vet. Parasitol. 211, 234–240.

Bailey, W.S., 1972. *Spirocerca lupi*: a continuing inquiry. J. Parasitol. 58, 3–22.

Bailey, W.S., 1963. Parasites and cancer: sarcoma in dogs associated with *Spirocerca lupi*. Ann. N. Y. Acad. Sci. 108, 890–923.

Bailey, W.S., Cabrera, D.J., Diamond, D.L., 1963. Beetles of the Family *Scarabaeidae* as intermediate hosts for *Spirocerca lupi*. J. Parasitol. 49, 485–488.

Ballesteros, F., 1998. Las especies de caza en España: biología, ecología y conservación. Colección técnica. Oviedo, 316 pp.

Barbour, A.D., Pugliese, A., 2000. On the Variance-to-Mean Ratio in Models of Parasite Distributions. Adv. Appl. Probab. 32, 701–719.

Bozick, B.A., Real, L.A., 2015. Integrating parasites and pathogens into the study of geographic range limits. Q. Rev. Biol. 90, 361–380.

Brodey, R.S., Thompson, R.G., Sayer, P.D., Eugster, B., 1977. *Spirocerca lupi* infection in dogs in Kenya. Vet. Parasitol. 3, 49–59.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own term: Margolis et al. revisited. J. Parasitol. 83, 575–583.

Calero-Bernal, R. Gómez-Gordo, L., Corchado-Araujo, M. Cuesta-Gerveno, J.M. Blanco-Ciudad, J., Pérez-Martín, J.E., 2011. Prevalence of *Spirocerca lupi* in red foxes from Extremadura (Spain). XII Congreso Ibérico de Parasitología. Zaragoza, Spain.

Čabanová, V., Miterpáková, M., Druga, M., Hurníková, Z., Valentová, D., 2018. GIS-based environmental analysis of fox and canine lungworm distribution: an epidemiological study of *Angiostrongylus vasorum* and *Crenosoma vulpis* in red foxes from Slovakia. Parasitol. Res. 117 , 521–530.

Carvalho, J.C., Gomes, P., 2004. Feeding resource partitioning among four sympatric carnivores in the Peneda-Gerês National Park (Portugal). J. Zool. 263, 275–283.

Dell'Arte, G.L., Laaksonen, T., Norrdahl, K., Korpimäki, E., 2007. Variation in the diet composition of a generalist predator, the red fox, in relation to season and density of main prey. Acta Oecol. 31, 276-281.

Diakou, A., Karamanavi, E., Eberhard, M., Kaldrimidou, E., 2012. First report of *Spirocerca lupi* infection in red fox *Vulpes vulpes* in Greece. Wildl. Biol. 18, 333-336.

Dixon, K., McCue, J.F., 1967. Further observations on the epidemiology of *Spirocerca lupi* in the Southeastern United States. J. Parasitol. 53, 1074–1075.

Environmental Systems Research Institute (ESRI), 2017. ArcGIS Release 10.6.

Eira, C., Viganda, J., Torres, J., Miquel, J., 2006. The helminth community of the red fox, *Vulpes vulpes*, in Dunas de Mira (Portugal) and its effect on host condition. Wildl. Biol. Pract. 2, 26–36.

Evangelista, P.H., Kumar, S., Stohlgren, T.J., Jarnevich, C.S., Crall, A.W., Norman III, J.B., Barnett, D.T., 2008. Modelling invasion for a habitat generalist and a specialist plant species. Divers. Distrib. 14, 808–817.

Ferrantelli, V., Riili, S., Vicari, D., Percipalle, M., Chetta, M., Monteverde, V., Gaglio, G., Giardina, G., Usai, F., Poglayen, G., 2010. *Spirocerca lupi* isolated from gastric lesions in foxes (*Vulpes vulpes*) in Sicily (Italy). Polish Journal of Veterinary Sciences 13, 465-471.

Fourcade, Y., Engler, J.O., Rödder, D., Secondi, J., 2014. Mapping species distributions with MAXENT using a geographically biased sample of presence data: A performance assessment of methods for correcting sampling bias. PLoS One 9, 1–13.

González, J., García-Marín, J.F., Muñoz, M., Martínez-Nistal, J.J., Pérez, V., Ferreras, M., 2009. Hallazgos anatomopatológicos en zorros (*Vulpes vulpes*) de Castilla y León. XXI Reunión de la SEAPV.

Gortázar, C., 2007. *Vulpes vulpes*. Zorro. In: Palomo, L.J., Gisbert, J., Blanco, J.C. (Eds.), Atlas y Libro Rojo de los Mamíferos Terrestres de España. Dirección General para la Biodiversidad-SECEM-SECEMU, Madrid, pp. 277-279.

Gortázar, C., Villafuerte, R., Lucientes, J., Fernández de Luco, D., 1998. Habitat related differences in helminth parasites of red foxes in the Ebro Valley. Vet. Parasitol. 80, 75–81.

Heiberger, R.M., 2018. HH: Statistical Analysis and Data Display: Heiberger and Holland. R package version 3.1-35.

Huang, S., Bininda-Emonds, O.R., Stephens, P.R., Gittleman, J.L., Altizer, S., 2014. Phylogenetically related and ecologically similar carnivores harbour similar parasite assemblages. J. Anim. Ecol. 83, 671–680.

Jiménez, J., Monsalve, M.A., Raga, J.A., 2012. Mamíferos de la Comunitat Valenciana. Colección Biodiversidad, 19. Conselleria d'Infraestructures, Territori i Medi Ambient. Generalitat Valenciana, Valencia.

Joubert, K.E., McReynolds, M.J., Strydom, F., 2005. Acute aortic rupture in a dog with spirocercosis following the administration of medetomidine. J. S. Afr. Vet. Assoc. 76, 159–162.

Le Sueur, C., Bour, S., Schaper, R., 2010. Efficacy of a combination of imidacloprid 10%/moxidectin 2.5% spot-on (Advocate® for dogs) in the prevention of canine spirocercosis (*Spirocerca lupi*). Parasitol. Res. 107, 1463–1469.

Lester, R.R.J., 1984. A review of methods for estimating mortality due to parasites in wild fish populations. In: Helgoländer Meeresuntersuchungen. Springer-Verlag, Hamburg, pp. 53–64.

Lobetti, R.G., 2000. Survey of the incidence, diagnosis, clinical manifestations and treatment of *Spirocerca lupi* in South Africa. J. S. Afr. Vet. Assoc. 71, 43–46.

Maksimov, P., Hermosilla, C., Taubert, A., Staubach, C., Sauter-Louis, C., Conraths, F.J., Vrhovec, M.G., Pantchev, N., 2017. GIS-supported epidemiological analysis on canine *Angiostrongylus vasorum* and *Crenosoma vulpis* infections in Germany. Parasit. Vectors. 10, 108.

Mazaki-Tovi, M., Baneth, G., Aroch, I., Harrus, S., Kass, P.H., Ben-Ari, T., Zur, G., Aizenberg, I., Bark, H., Lavy, E., 2002. Canine spirocercosis: clinical, diagnostic, pathologic, and epidemiologic characteristics. Vet. Parasitol. 107, 235–250.

Magi, M., Guardone, L., Prati, M.C., Mignone, W., Macchioni, F., 2014. Extraintestinal nematodes of the red fox *Vulpes vulpes* in north-West Italy. J. Helminthol. 89, 506–511.

Morandi, F., Angelico, G., Verin, R., Gavaudan, S., 2014. Fatal spirocercosis in a free-ranging red fox. Vet. Rec. 174, 228. doi: 10.1136/vr.g1802.

Mylonakis, M.E., Koutinas, A.F., Liapi, M.V., Saridomichelakis, M.N., Rallis, T.S., 2001. A comparison of the prevalence of *Spirocerca lupi* in three groups of dogs with different life and hunting styles. J. Helminthol. 75, 359–361.

Oliveira-Sequeira, T.C.G., Amarante, A.F.T., Ferrari, T.B., Nunes, L.C., 2002. Prevalence of intestinal parasites in dogs from São Paulo State, Brazil. Vet. Parasitol. 103, 19–27.

Pavlović, I., Kulišić, Z., Milutinović, M., 1997. The role of foxes (*Vulpes vulpes* L.) in the epizootiology and epidemiology of nematode parasitic zoonozes. Acta Vet. 47, 177-182.

Phillips, S.J., 2017. A Brief Tutorial on Maxent. http://biodiversityinformatics.amnh.org/open_source/maxent/.

Piqueras, J., 1999. El espacio valenciano: una síntesis geográfica. Editorial Gules, Valencia.

Poulin, R., Morand, S, 2000. The diversity of parasites. Q. Rev. Biol., 75, 277–293.

POULIN, R., 2007. Are there general laws in parasite ecology? Parasitology, 134, 763–776

R Core Team, 2018. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Radosavljevic, A., Anderson, R.P., 2014. Making better Maxent models of species distributions: Complexity, overfitting and evaluation. J. Biogeogr. 41, 629–643.

Ramachandran, P.V., Shakir, S.A., Ramakrishnan, R., 1984. Spirocercosis in canines – a necropsy survey. Cheiron Tamil Nadu. J. Vet. Sci. Anim. Husb. 13, 132–135.

Reche-Emonot, M., Beugnet, F., Bourdoiseau, G., 2001. Étude épidémiologique et clinique de la spirocercose canine à l'Île de La Réunion à partir de 120 cas. Rev. Méd. Vét. 152, 469–477.

Rinas, M., Nesnek, R., Kinsella, M., DeMatteo, K., 2009. Fatal aortic aneurysm and rupture in a neotropical bush dog (*Speothos venaticus*) caused by *Spirocerca lupi*. Vet. Parasitol. 164, 347–349.

Rothmann, W., de Waal, P.J., 2017. Diversity of *Spirocerca lupi* in domestic dogs and black-backed jackals (*Canis mesomelas*) from South Africa. Vet. Parasitol. 244, 59–63.

Rojas, A., Dvir, E., Farkas, R., Sarma, K., Borthakur, S., Jabbar, A., Markovics, A., Otranto, D., Baneth, G., 2018a. Phylogenetic analysis of *Spirocerca lupi* and *Spirocerca vulpis* reveal high genetic diversity and intra-individual variation. Parasit. Vectors. 11, 639.

Rojas, A., Sanchis-Monsonís, G., Alić, A., Hodžić, A., Otranto, D., Yasur-Landau, D., Martínez-Carrasco, C., Baneth, G., 2018b. *Spirocerca vulpis sp. nov*. (Spiruridae: *Spirocercidae*): description of a new nematode species of the red fox, *Vulpes vulpes* (Carnivora: *Canidae*). Parasitology 145, 1917–1928.

Rózsa, L., Reiczigel, J., Majoros, G., 2000. Quantifying parasites in samples of hosts. J. Parasitol. 86, 228–232.

Saenz de Buruaga, M., Lucio, A.J., Purroy, F.J., 1991. Reconocimiento de sexo y edad en especies cinegéticas. Gobierno Vasco, Vitoria. 127 pp. Diputación Foral de Álava.

Sanchis-Monsonís, G., 2015. Parasitofauna del zorro (*Vulpes vulpes*) en la Comunidad Valenciana. Universidad de Murcia.

Sándor, A.D., D'Amico, G., Gherman, C.M., Dumitrache, M.O., Domșa, C., Mihalca, A.D., 2017. Mesocarnivores and macroparasites: altitude and land use predict the ticks occurring on red foxes (*Vulpes vulpes*). Parasit. Vectors 10, 173.

Sarmento, P., Cruz, J., Eira, C., Fonseca, C., 2009. Evaluation of camera trapping for estimating red fox abundance. J. Wildl. Manage. 73, 1207-1212.

Segovia, J., Miquel, J., Feliu, C., Torres, J., 2001. Morphological and morphometric study of *Spirocerca lupi*. Helminthologia 38, 115-121.

Segovia, J.M., Torres, J., Miquel, J., 2004. Helminth parasites of the red fox (*Vulpes vulpes* L., 1758) in the Iberian Peninsula: An ecological study. Acta Parasitol. 49, 67–79.

Sen, K., Anantaraman, M., 1971. Some observations on the development of *Spirocerca lupi* in its intermediate and definitive hosts. J. Helminthol. 45, 123–131.

Shaw, D.J., Grenfell, B.T., Dobson, A.P., 1998. Patterns of macroparasite aggregation in wildlife host populations. Parasitology 117, 597–610.

Shimalov, V.V., Shimalov, V.T., 2003. Helminth fauna of the red fox (*Vulpes vulpes* L. 1758) in Southern Belarus. Parasitol. Res. 89, 77–78.

Valcárcel, F. González, J. Aguilar, A. Sánchez, M. González, M.G. Suárez, R. Tercero, A.M. Tercero, J.M. Nieto, J.M. González-Guirado, A.M. Olmeda, A.S., 2018. Spirocercosis in red fox (*Vulpes vulpes*) in a natural reserve located in a meso-Mediterranean area, Veterinary Parasitology: Regional Studies and Reports 13, 115-119.

Vale, P.F., Choisy, M., Little, T.J., 2013. Host nutrition alters the variance in parasite transmission potential Biol. Lett. 9, 20121145.

Van der Merwe, L.L., Kirberger, R.M., Clift, S., Williams, M., Keller, N., Naidoo, V., 2008. *Spirocerca lupi* infection in the dog: a review. Vet. J. 176, 294–309.

Warren, D.L., Glor, R.E., Turelli, M., 2010. ENMTools: a toolbox for comparative studies of environmental niche models. Ecography (Cop.). 33, 607–611.

Dataset	Description	Source	Resolution
DEM	Digital elevation model describing the altitude for each pixel	Instituto Geográfico Nacional (España) http://www.ign.es/web/ign/portal)	10 metre
Slope	Raster describing the slope of each pixel	Derived from DEM	
Latitude	Raster describing the latitude in UTM coordinates of each pixel	Derived from DEM	
Longitude	Raster describing the longitude in UTM coordinates of each pixel	Derived from DEM	
NDVI (WP)	Raster describing the average Normalized Difference Vegetation Index value during the wet period for each pixel	MOD13A2 V.6 (2013) https://modis-land.gsfc.nasa.gov/	1 kilometre
NDVI (DP)	Raster describing the average Normalized Difference Vegetation Index value during the dry period for each pixel		
Precipitation (WP)	Raster describing the average precipitation (mm rain) during the wet period for each pixel	WorldClim V.2 http://www.worldclim.org/	1 kilometre
Precipitation (DP)	Raster describing the average precipitation (mm rain) during the dry period for each pixel		
T max (WP)	Raster describing the maximum temperature (degrees) during the wet period for each pixel		
T max (DP)	Raster describing the maximum temperature (degrees) during the dry period for each pixel		
T min (WP)	Raster describing the minimum temperature (degrees) during the wet period for each pixel		
T min (DP)	Raster describing the minimum temperature (degrees) during the wet period for each pixel		
T average (WP)	Raster describing the average temperature (degrees) during the wet period for each pixel		
T average (DP)	Raster describing the average temperature (degrees) during the wet period for each pixel		
Rivers distance	Raster describing the distance from the closest river (in kilometre) for each pixel	DIVA-GIS https://www.diva-gis.org/	Rasterized to 10 metre
Distance from urban areas	Raster describing the distance from the closest urban area (in kilometre) for each pixel	Derived from CORINE Land Cover (CLC)	10 metre
Distance from wetlands	Raster describing the distance from the closest wetland area (in kilometre) for each pixel	Derived from Valencia shapefile retrived from DIVA-GIS https://www.diva-gis.org/	Rasterized to 1kilometre

Table 1. Original environmental variables from Valencian Community tested for collinearity.

				Total (n=286)					
	Prevalence				***Abundance***			***Intensity***	
Positive	P(%)	IC 95%	Total adult parasites	x̄	Variance	Variance-to-mean ratio	Median	IQR	Range
63	22.0	17.4-27.3	605	2.1	41	0.05	5	11	43
				Adult (n=225					
	Prevalence				*Abundance*			*Intensity*	
Positive	P(%)	IC 95%	Total adult parasites	x̄	Variance	Variance-to-mean ratio	Median	IQR	Range
62	27.4	21.6-33.2	604	2.7	50.7	0.05	5	11	43
				Juvenile (n=61)					
	Prevalence				*Abundance*			*Intensity*	
Positive	P(%)	IC 95%	Total	x̄	Variance	Variance-to-mean ratio	Median	IQR	Range
1	1.6%	-1.5-4.8	1	-	-	-	-	-	-
				Male (n=152)					
	Prevalence				*Abundance*			*Intensity*	
Positive	P(%)	IC 95%	Total adult parasites	x̄	Variance	Variance-to-mean ratio	Median	IQR	Range
29	19.0	12.8-25.3	300	2	40	0.05	6	11	43
				Female (n=135)					
	Prevalence				***Abundance***			***Intensity***	
Positive	P(%)	IC 95%	Total adult parasites	x̄	Variance	Variance-to-mean ratio	Median	IQR	Range
34	25.1	17.8-32.5	305	2.6	42.8	0.06	4	8.5	43

Table 2. Prevalence, abundance and intensity of *Spirocerca vulpis* in red foxes from Valencian Community (SE Spain).

Dataset	Permutation importance (%)
DEM	4.2
Latitude	3.6
Longitude	3.6
NDVI (WP)	1.9
NDVI (DP)	1.2
Precipitation (WP)	0
Precipitation (DP)	0
T min (WP)	51.5
T min (DP)	0
T average (WP)	0
T average (DP)	33.9

Note. The contribution for each variable is determined by randomly permuting the values of that variable among the training points (both presence and background) and measuring the resulting decrease in training AUC. Values are normalized to give percentages

Table 3. Relative contributions of the environmental variables to the model.

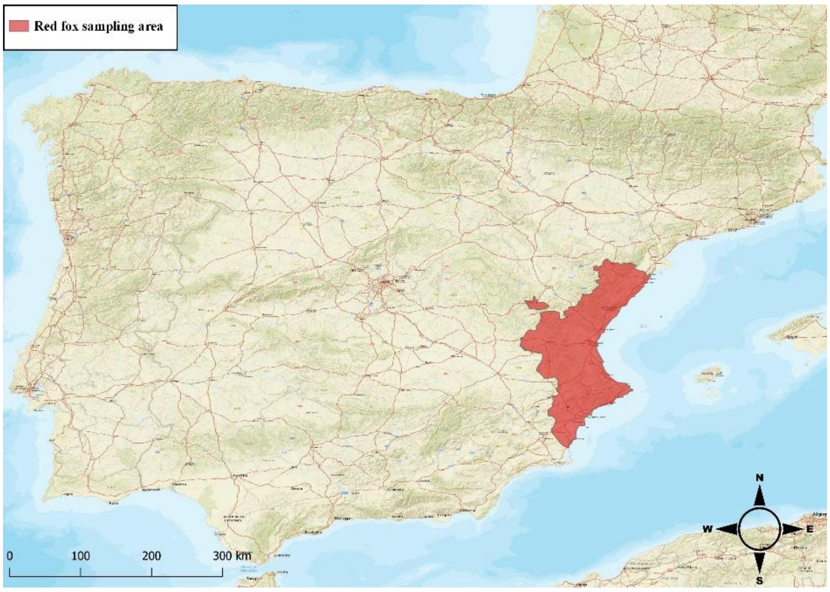

Figure 1. Location of the Valencian Community (SE Spain) in the Iberian Peninsula.

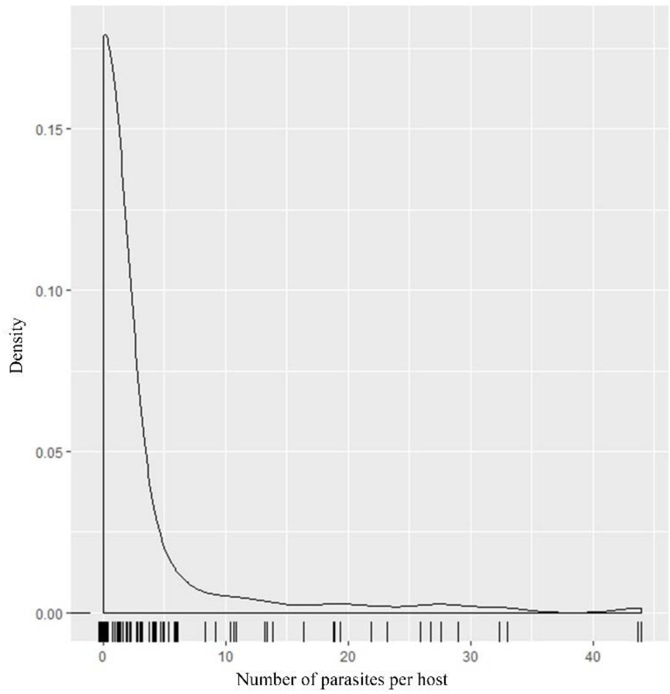

Figure 2. Density plot of *Spirocerca vulpis* distribution in red foxes from the Valencian Community (SE Spain).

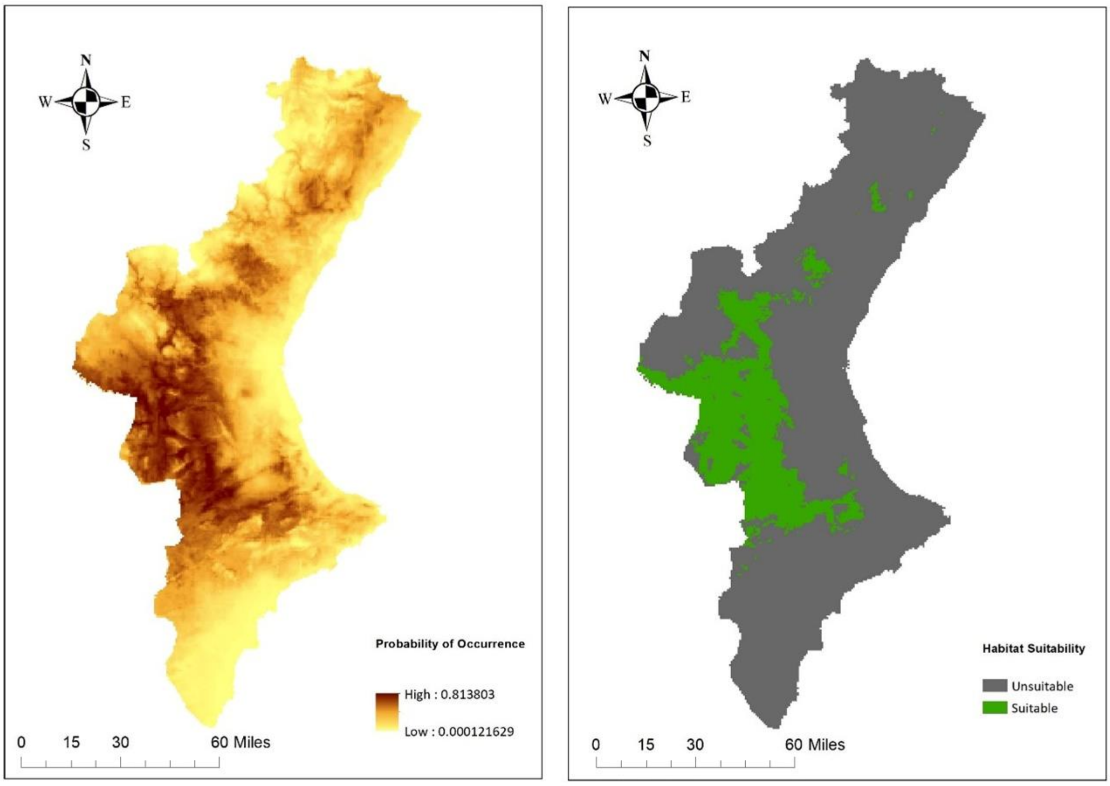

Figure 3. *Spirocerca vulpis* occurrence in foxes and habitat suitability in Valencian Community (SE Spain).

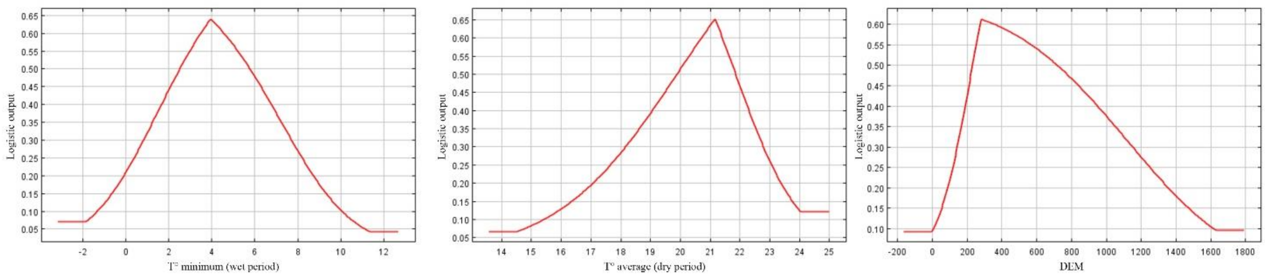

Figure 4. Response curves representing the probability of *Spirocerca vulpis* presence for Temperature minimum (wet period), Temperature average (dry period) -both expressed as degrees Celsius-, and DEM (Digital elevation model) -expressed as metres above sea level-.