Parasites in red-fox: the environmental risk factors (Valencia, Spain)

Gloria Sanchis Monsonis[1] , ***Angela Fanelli***[2], Carlos Martinez-Carrasco[1], Paolo Tizzani[3]
(1) Dpto. Sanidad Animal. Facultad de Veterinaria. Universidad de Murcia. Campus Universitario s/n. 30100 Espinardo, Murcia. España.
(2) Indipendent Researcher
(3) Dipartimento di Scienze Veterinarie, Università degli studi di Torino, Largo Paolo Braccini 2, 10095, Grugliasco (Torino).

ABSTRACT
Climate and human driven environmental changes, are threatening biodiversity and modifying the distribution of pathogens. The monitoring of red fox (*Vulpes vulpes*) is especially interesting to monitor ecosystem health, as it is the reservoir of numerous pathogens. This study investigates the factors influencing the presence of nematode species (*Spirocerca vulpis, Angiostrongilus vasorum, Crenosoma vulpis, Toxocara canis* and *Toxascaris leonina*) parasitizing red fox in the Valencian Community (south-east Spain) from 2006 to 2013. Maxent software was used to model and predict parasite distributions. Continuous and discrete prediction maps were built using ArcMap 10.6. Models performed differently, depending on the parasite species. We found an accurate spatial pattern for *S. vulpis*, while the models for other nematodes had less predictive power. Despite the difference in performance, the distribution of parasites was mainly influenced by climatic variables. Considering the role of red fox in the maintenance and dispersion of a wide range of pathogens, we encourage future researches to further investigate its role as health risk factor for animals and humans.
KEYWORDS: fox, parasites, climate

1. INTRODUCTION

The red fox is among the carnivores with broader range, able to adapt to a wide variety of habitats and climatic conditions (Scott et al., 2014). Red foxes are definitive hosts for many parasites, including zoonotic pathogens (i.e. *Echinococcus multilocularis*, *T.canis*), representing a health risk for animals and humans (Gicik et al., 2009). Geographic distribution of foxes' parasites is poorly known in the Valencian Community, and predictive habitat distribution models may fill this gap. Several species distribution models (SDM) exist and, among them, Maximum Entropy (MAXENT) has become popular in recent years. Maxent algorithm relates the locations of species with environmental predictors, estimating their response function and contribution, as well as the probability of species presence (Fourcade et al., 2014).

2. GOAL

Considering the above, we used presence-only data on the occurrence of parasites in foxes to i) evaluate the environmental variables influencing the distribution of parasites and ii) map the areas at risk for parasites presence.

3. SOLUTION: METHODS

Data on red fox**:** data were collected in the context of a surveillance program carried out in the Valencian Community between 2006 and 2013. Two hundred and eighty-seven foxes, were analysed. Based on parasitological examination, a database on the occurrence of five nematode species (*A. vasorum, S. vulpis, T.canis, T.leonina, Crenosoma vulpis*) was created.
Environmental data: 17 environmental variables were used to build the predictive models, including (table 1):

Dataset	Source	Original resolution
DEM	Instituto Geográfico Nacional (España)	10 metre
Slope	Derived from DEM	
Latitude	Derived from DEM	
Longitude	Derived from DEM	
NDVI (WP)	MOD13A2 V.6 (2013)	1 kilometre
NDVI (DP)		
Precipitation (WP)	WorldClim V.2	1 kilometre
Precipitation (DP)		

T max (WP)		
T max (DP)		
T min (WP)		
T min (DP)		
T average (WP)		
T average (DP)		
Rivers distance	DIVA-GIS	Rasterize to 10 metre
Distance from urban areas	Derived from CORINE Land Cover (CLC)	Rasterize to 10 metre
Distance from wet lands	Ramsar Sites	Rasterize to 10 metre

Table 1. Environmental variables used to model parasite's distribution.

The monthly values of climatic and 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 reprojected using the same CRS (EPSG: 3042). This process was done using ArcMap 10.6.

Epidemiological indexes and explorative analysis**:** parasitic community was evaluated by mean of the epidemiological indexes of prevalence (positive/total animals), abundance (number parasites / total animals) and intensity (number parasites / positive animals). Records of presence of each nematode were incorporated into a geographic information system (GIS) (ArcMap 10.6) and the parasite richness was displayed by mean of a heatmap, using the "point density tool" function (http://desktop.arcgis.com/en/arcmap/10.3/tools/spatial-analyst-toolbox/point-density.htm).

Models creation and selection**:** the HH package (R v3.5.0) was used to compute variance inflation factors (VIFs) and evaluate collinearity among the independent variables. Variables with VIF < 10 were excluded from the model. Models were built using a backward selection approach in Maxent v3.4.1. For each parasite, the most parsimonious model was selected using ENMTools v1.3 to compute the Akaike Information Criterion (AIC). Variables were removed based on the jacknife test (lower contribution to AUC). Maxent 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). Maximum training sensitivity plus specificity was selected as threshold, to convert continuous prediction (logistic) into binary output.

Variable Importance and model performance**:** permutation importance (PI) was used to assess the contribution of each factor. PI measures the model decrease in quality, when the variable is not included. Response curves were generated to display the relationship of the environmental factors with the probability of parasite presence. To assess performance of the model, area under the curve value (AUC) was used. A model that performs no better than random will have an AUC of 0.5, whereas a model with perfect discrimination would have an AUC of 1. Maxent output provides also the "training gain" parameter which describes how much better the distribution fits the presence data compared to a uniform distribution.

4. IMPACT: RESULTS AND DISCUSSIONS

Epidemiological Indexes**:** nematode's epidemiological indexes are reported in table 2.

	C. vulpis	***A. vasorum***	***S. vulpis***	***T. canis***	***T. leonina***
Prevalence	28% [27.6-28.4]	40.4% [40.1-40.7]	22% [21.7-22.3]	27%[26.5-27.5]	25% [24.7-25.3]
Abundance	1.8 [1.2-2.4]	7.5[5.3-9.7]	2.1 [1.4-2.8]	0.8 [0.6-1.0]	2.9 [1.8-4.0]
Intensity	6.3 [4.3-8.3]	18.7[13.6-23.8]	9.6[6.9-12.3]	7.8[7.2-8.4]	11.7 [7.6-15.8]

Table 2. Prevalence, abundance and intensity of the nematode species. Values are provided with confidence intervals

The highest prevalence was detected for *A. vasorum*, and the lowest one for *S. vulpis*. *A. vasorum* prevalence is in line with values found in Northern Europe, and indicates that the Valencian Community presents optimal environmental characteristic for this parasite. The prevalence of *C. vulpis*, as well, is among the highest found in Spain (Segovia et al., 2004). Abundance and intensity show similar patterns, being still *A. vasorum*, the species with the highest values. Parasite aggregation, represented by mean of a heatmap (figure 1) highlighted clusters in the northern and central part of the Region. This areas represents the part of Valencia Community with less “extreme” climatic conditions.

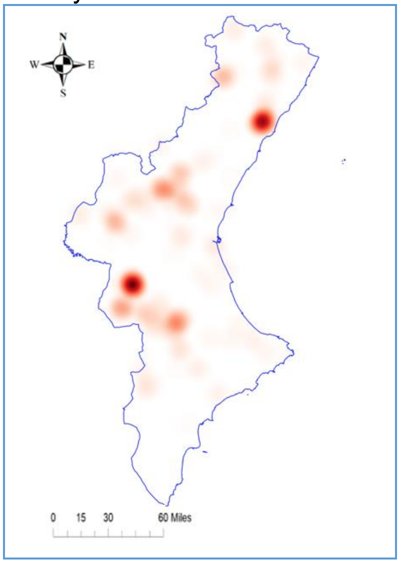

Figure 1. Heat map: distribution of parasitic cluster in the study area

<u>Model results</u>**:** table 3 summarizes the model performances, in terms of AUC and training gain. The final model for each nematode is presented in figure 2. The only model with acceptable performance was the one for *S. vulpis*, while the others showed AUC values close to 0.6 (low predictive performance). For this reason, the results will focalize mainly on *S. lupi*.

Species	AUC	Training Gain
A. vasorum	0.63	0.147
C. vulpis	0.62	0.268
S. vulpis	0.91	0.489
T. canis	0.59	0.119
T. leonina	0.65	0.321

Table 3. Final model for each nematode species, AUC and training gain values are reported.

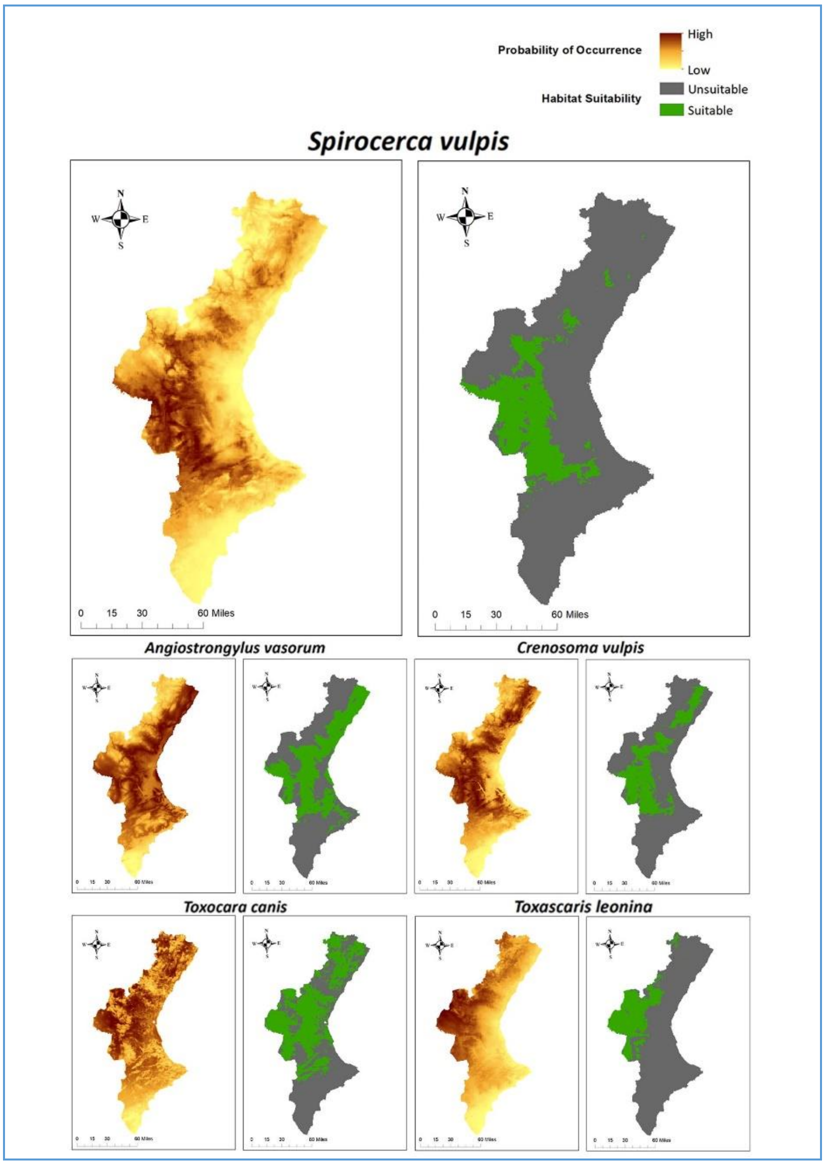

Figure 2. Predictive models for the five nematode species. Continuous and binary output are reported.

Spirocerca vulpis.

The predictive accuracy of *S. vulpis* model was very high (AUC=0.91). 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 1).

Among the factors influencing its distribution, the minimum temperature of WP had the highest permutation importance (PI=51.5), followed by the average temperature of DP (PI= 33.9) and the altitude (PI= 4.2). Probability of presence of the parasite increases from -2 degree and maximize at 4 degrees in WP, while in DP it drops above 21 degrees. Regarding altitude, the model identifies optimal condition for parasite presence around 300 meters a.s.l. The three variables explain 90% of the prediction accuracy of the model. The remaining part is mainly due to latitude (PI=3.6) and longitude (PI=3.6).

The two first predictors highlight the key role that climate plays on the presence of paratenic and intermediate hosts, and so on the presence of *S. vulpis* (Bailey, 1972). The effect of altitude on distribution of parasites is also significant, and it is probably linked to higher fox densities at low altitudes (Sandor et al., 2017). As shown in figure 2, the presence of this nematode is restricted to limited areas. This pattern is confirmed by previous studies in dogs, which described the spirocercosis as an endemic disease in well-defined areas (i.e. Mazaki-Tovi et al., 2002). The presence of this parasites in clustered location, is also highlighted by the significant role of latitude and longitude in the models, that presents a peak in a very narrow latitudinal and longitudinal range.

Angiostrongylus vasorum.
The average temperature (DP) (PI= 67.7) and precipitation (DP) (PI= 6.2) have the greatest contribution. Outbreaks of this nematode are in fact associated with wet and temperate years, when intermediate gastropod hosts are more abundant (Cobb and Fisher, 1990). Again, the factors that influence most the parasite distribution are the climatic ones, directly influencing the larval survival and the intermediate host presence (Jeffery et al., 2004).
Crenosoma vulpis
The most important explanatory variables were again climatic: average temperature (DP) (PI= 33.5), and minimum temperature (WP) (PI= 29.7). As for *A. vasorum*, this relationship can be explained by the larval sensitivity to high temperature, and the need of humidity for the intermediate host presence.
Toxocara canis
The model for this species is the less accurate. This result may be due to the fact that *T. canis* is transmitted by ingestion of larvated eggs and by vertical via to the puppies (lower influence of environmental factors). It is worthy to highlight that this model was the only one in which the distance from urban areas was retained, with higher probability of occurrence close to urban areas (figure 3). This finding, along with the high prevalence of *T. canis* (27%), is of particular interest from a public health perspective (higher risk of human infection in urban and periurban areas).

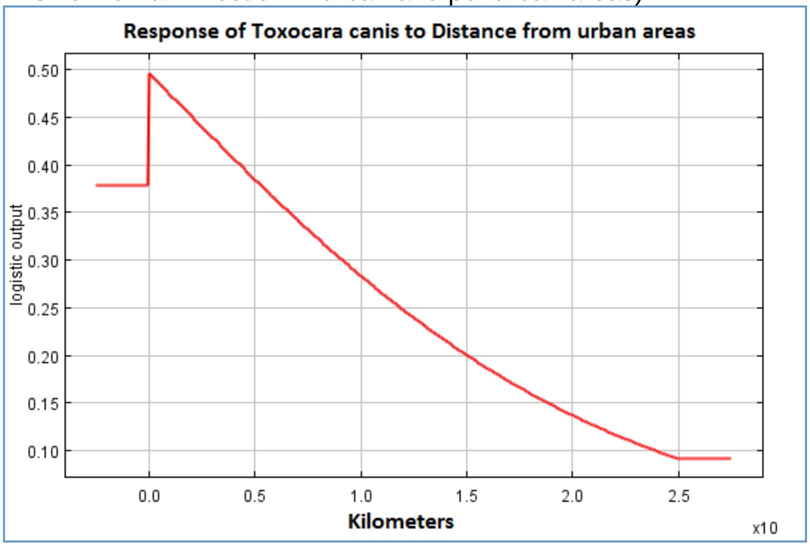

Figure 3. Response of T. canis to distance from urban areas

Toxascaris leonina
The environmental variable with the highest permutation importance (PI= 54.9) was the minimum temperature (WP), with decreasing probability of presence with increasing temperature value.

Our study highlighted that red fox is a carrier of nematodes whose epidemiological role is noticeable, either because of their zoonotic potential (*T.canis*) or their pathogenicity for domestic animals (*A.vasorum*, *S. vulpis*). In this context, predictive models are important tools that allow to understand the factor influencing parasite distribution, and map the risk of disease transmission. To the best of our knowledge, this is the first study describing environmental risk factors associated to nematodes occurrence in the Valencian Community.
The application of Maxent algorithm provides valuable insights on the relationship between parasite presence and predictors. Climatic variables were the main factors selected in all the models, demonstrating their importance on parasite distribution, and the impact that climate changes may have on changing risk of disease transmission. 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. Many studies have emphasised the causal relationship between climate change and parasitic diseases (i.e. Patz et al., 2000). Among infectious diseases,

helminthiases are important because of their impact on human and animal health and their capacity to regulate the abundance of wild animal populations, and hence to affect the functioning of ecosystems.
On the other hand, in our study, the only parasite that was influenced by human mediate variables was *T. canis* (more likely to appear close to urban areas). This finding highlights the potential threat of red foxes for public health.
We found a significant difference in accuracy and reliability among the nematode species models. *S. vulpis* model performed very well, while the others showed low performances. This may be due to several reasons: a) data resolution might affect the explanatory power and predictive accuracy; b) generalist species, as *T. canis*, adapts to a wide range of environmental conditions, not easily defined by independent variables; c) variable selection may influence the quality of the model. A different set of variables may improve the discriminatory capacity. Further modelling on parasite distribution, taking into consideration multidimensional approach and different environmental variables could be valuable to refine the predictive models.

5. BIBLIOGRAPHY

- Bailey WS (1972). *Spirocerca lupi*: a continuing inquiry. *The Journal of parasitology*, 58 (1), 3-22
- Cobb MA, Fisher MA (1990). Angiostrongylus vasorum: transmission in southeast England. *Veterinary Record*, 126 (21).
- Fourcade Y, Engler JO, 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(5): e97122
- Gicik Y, Kara M, Sari B, Kiliç K, Arslan Mö. (2009). Intestinal Parasites of Red Foxes (*Vulpes vulpes*) and Their Zoonotic Importance for Humans in Kars Province. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 15 (1): 135-140.
- Jeffery RA, Lankester MW, McGrath MJ, Whitney HG (2004). *Angiostrongylus vasorum and Crenosoma vulpis* in red foxes (*Vulpes vulpes*) in Newfoundland, Canada. *Canadian Journal of Zoology*, 82 (1), 66-74.
- Mazaki-Tovi M, Baneth G, Aroch I, Harrus S, Kass PH, Ben-Ari T, Zur G, Aizenberg I, Bark H, Lavy E (2002). Canine spirocercosis: clinical, diagnostic, pathologic, and epidemiologic characteristics. *Veterinary Parasitology*, 107 (3), 235-250.
- Patz JA, Graczyk TK, Geller N, Vittor AY (2000). Effect of environmental change on emerging parasitic diseases. Int. J. Parasitol., 30, 1395-1405.
- Radosavljevic A, Anderson RP (2014) Making better MAXENT models of species distributions: complexity, overfitting and evaluation. Journal of Biogeography, 41, 629–643
- Sándor AD, D'Amico G, Gherman CM, Dumitrache MO, Domșa C, Mihalca AD (2017). Mesocarnivores and macroparasites: altitude and land use predict the ticks occurring on red foxes (Vulpes vulpes). *Parasites & vectors*, *10*(1), 173.
- Scott DM, Berg MJ, Tolhurst BA, Chauvenet AL, Smith GC, Neaves K, Lochhead J, Baker PJ (2014). Changes in the distribution of red foxes (*Vulpes vulpes*) in urban areas in Great Britain: findings and limitations of a media-driven nationwide survey. *PloS ONE*, 9 (6).
- Segovia JM, Torres J, Miquel J (2004). Helminth parasites of the red fox (Vulpes vulpes L., 1758) in the Iberian Peninsula: an ecological study. *Acta Parasitologica*, 49: 67-79.