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Monthly analysis of the current risk of heartworm transmission in Portugal and Spain through ecological niche modeling as a control measure

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ABSTRACT

Heartworm disease (cardiopulmonary dirofilariosis) is a vector-borne parasitic disease caused by the parasitic nematode Dirofilaria immitis. It is transmitted by different species of culicid mosquitoes, with Culex pipiens being the most important species in the Iberian Peninsula, and Culex theileri in the Canary Islands and Madeira. The development of risk maps using ecological niche models (ENMs) has established itself as a useful tool in the prevention and control of various parasitic infections in different territories. The aim of this study was to produce monthly infection risk maps for all territories in Spain and Portugal, based on ecological modelling of Cx. pipiens in the Iberian Peninsula, the Islands and Madeira, and of Cx. pipiens together with Cx. theileri in the Canary Islands. These models were weighted with the number of generations of D. immitis calculated each month, thus obtaining infection risk maps for each month of the year. Models indicate that the risk of transmission is highest in summer, gradually decreasing in autumn until reaching zero levels in winter in most territories. In the Iberian Peninsula, the most affected areas are the south, the Mediterranean coast and the Balearic Islands. In the Canary Islands, the risk remains moderate during winter due to its thermal stability, especially in densely populated coastal areas. A similar pattern is observed in the Azores Islands and Madeira, where the risk, although lower, persists in winter and is concentrated in low-lying, inhabited areas. This approach allows the dynamics of heartworm infection to be studied throughout the year and constitutes the first time that monthly risk models have been developed in these territories, as well as the first risk maps for D. immitis transmission in the Azores Islands and Madeira.

1. Introduction

Spain

Heartworm disease is a vector-borne zoonosis caused by the parasitic nematode *Dirofilaria immitis*. Its definitive hosts are mainly domestic and wild dogs, which act as reservoirs, although it can also affect felids and other carnivores. Humans can be affected accidentally, but in most cases the infection is benign and asymptomatic (Simón et al., 2012; Morchón et al., 2022).

Culicidae mosquitoes act as vectors of the parasite. They ingest microfilariae (first-stage larvae, L1) when feeding on peripheral blood from an infected host. The microfilariae develop into third-stage larvae (L3) after two moults in approximately 14 days at average outdoor temperatures and high humidity, with the moulting period being shorter at higher temperatures and longer at lower temperatures. The infective L3 larvae are transmitted to the definitive host when the vectors feed again on peripheral blood from another infected host (Simón et al.,

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2012)

Heartworm disease is distributed worldwide, although it is most prevalent in tropical and subtropical regions (Simón et al., 2012). As it has been reported in some territories, the infection prevalence depends on vector presence and climate conditions, which may vary seasonally across the year (Brown et al., 2012; Atkinson et al., 2025). In Europe, it is spreading from the traditionally endemic countries of the Mediterranean basin and the south-east of the continent to those in the central-northern areas, even reaching some regions near the Sea of Azov and the Black Sea (Morchón et al., 2022). In Spain and Portugal, countries in southern Europe, we observe a notable endemicity of the disease. An average prevalence of 6.47% and 5.90% has been reported in domestic dogs in Spain and Portugal, respectively (Montoya-Alonso et al., 2022; Esteves-Guimarães et al., 2024). The regions with the highest prevalence are located in the southern and north-western parts of the Iberian Peninsula and in the Balearic and Canary Islands, which are considered hyperendemic islands (Montoya-Alonso et al., 2022, 2024).

Parasite distribution has expanded from countries where the vector is established, such as southern European countries, to new locations not previously described as suitable for its establishment and development, such as northern countries, linking the establishment of the vector with that of the parasite (Morchón et al., 2022). In fact, in Europe, more than 25 species have been reported to act as vectors for heartworm infection (Morchón et al., 2022). In particular, in Portugal and Spain, *Culex pipiens* and *Culex theileri* are considered the most important species in the transmission of *D. immitis*, in addition to being the most abundant and widely distributed species. Both species are vectors of *D. immitis* in the Iberian Peninsula, and only *Cx. theileri* has been reported in the Canary Islands (Spain) and Madeira (Portugal) (Santa-Ana et al., 2006; Ferreira et al., 2015; Bravo-Barriga et al., 2016; Morchón et al., 2007, 2011).

Globalisation, the exchange of goods, the influence of climate change, and the increase in travel with infected animals acting as reservoirs are factors that could facilitate the introduction of new competent vectors in these regions (Simón et al., 2009, 2012; Dantas-Torres and Otranto, 2020). Their establishment is closely related to the presence of freshwater bodies, rivers, and irrigated crops, as well as anthropogenic and climatic factors such as humidity and temperature (Cancrini et al., 2007). Climatic factors such as temperature determine not only the potential distribution of the vectors but also the number of generations of the parasite within the vector (Morchón et al., 2023; Rodríguez-Escolar et al., 2023). Empirical studies have shown that minimum temperatures of 14 °C over constant periods during the life expectancy of the vector (≥ 10 days at optimum temperatures) are needed for the development of *D. immitis* larvae to occur (Fortin and Slocombe, 1981).

In relation to the control of vector-borne diseases, the use of ecological niche models (ENMs) stands out for obtaining colourimetric maps that analyse the risk of infection, which serve to implement prevention measures in animals and humans (Fleitas et al., 2022; Lawrence et al., 2023). These models are based on Geographic Information Systems (GIS) and focus on identifying areas where conditions are most favourable for the establishment of the vector based on the environmental requirements it needs to survive (Araújo and Guisan, 2006; Iwamura et al., 2020; Escobar, 2020; Omar et al., 2021; Velu et al., 2023; Wouters et al., 2024). In fact, recent studies have incorporated the behaviour of the parasite within the vector into these models, making it possible to generate infection risk maps that are closer to reality (Morchón et al., 2023; Rodríguez-Escolar et al., 2023, 2024a, 2024b, 2025; Genchi et al., 2024).

In Spain and Portugal, with the exception of the Azores Islands and Madeira, previous studies have been carried out in which maps have been generated to analyse the risk of infection with *D. immitis*, modelling the habitat suitability of *Cx. pipiens* and *Cx. theleri*, as well as the average number of generations of the parasite within the vector (Morchón et al., 2023; Rodríguez-Escolar et al., 2023). However, although their

usefulness is clear, these risk maps do not reflect monthly variations in risk based on the number of generations of *D. immitis* that occur each month, which is a limiting factor in these studies. For all these reasons, this study aims to analyse the risk of infection with *D. immitis*, taking into account monthly fluctuations of nematode generations across the entire peninsular territory of Spain and Portugal and their islands (Balearic Islands, Canary Islands), including, for the first time, the Azores Islands and Madeira. This new approach will allow for a more accurate description of the dynamics of parasite transmission and thus improve disease prevention and control strategies, especially in the context of vector expansion favoured by climate change and anthropogenic alterations to the environment.

2. Materials and methods

2.1. Study area

The study zones include the Iberian Peninsula and the Balearic Islands $(36^{\circ}00'-43^{\circ}47'N, 9^{\circ}30'W-4^{\circ}30'E)$, the Canary Islands $(27^{\circ}37'-29^{\circ}25'N,$ $13^{\circ}20'-18^{\circ}10'W)$, Madeira (32°38′-33°07′N, $16^{\circ}39'-17^{\circ}16'W$), and the Azores Islands $(36^{\circ}55'-39^{\circ}43'N,$ 24°46′-31°16′W) (Fig. 1). These are the southernmost territories of Europe and make up the countries of Spain and Portugal. The Iberian Peninsula is crossed by several rivers with large river basins, such as the Guadalquivir (south) and Ebro (north-east), and other smaller basins such as the Miño (north-west), Guadiana (south-west), Duero and Tajo (centre-west) and Segura (east). In the Balearic Islands, only the Cala Galdana River (Menorca) flows permanently; in the Azores Islands, the larger islands have numerous small streams, while in the Canary Islands and Madeira there are no rivers as such. The main mountain ranges on the peninsula are the Central System, the Iberian System, the Cantabrian Mountains, the Pyrenees, the Penibetic System and the Baetic System. The Canary Islands and Madeira are of very recent volcanic origin, with several phases of lava flows and typical volcanic relief, resulting in a very mountainous terrain. All the islands can be divided into three geographical areas: the mountains, the midlands, immediately below the peaks, and the coasts. The Azores Islands also have a volcanic origin associated with the convergence of the tectonic plates of America, Eurasia and Africa, with a lot of seismic and volcanic activity (DGRM, 2025; EU, 2025; Government of the Canary Islands, 2025; Maca-

The climate, according to the Köppen-Geiger climate classification, is highly varied in the study areas (Fig. 2). The Iberian Peninsula is dominated by a Mediterranean climate with different nuances. In general, the warm Mediterranean climate (Csa) dominates in coastal and low-lying areas, with dry, hot summers and mild winters. In Madeira and in the inland and elevated areas of the continent, the Mediterranean climate with mild summers (Csb) predominates, characterised by cooler summers and better distributed rainfall. In the north and north-west of the peninsula and in the Azores Islands, there is an oceanic climate (Cfb), with frequent rainfall and mild temperatures. The driest areas in the southeast have semi-arid climates, both cold (Bsk) and warm (Bsh), characterised by low rainfall and hot summers. In mountainous areas, the climate is continental, with cold winters and short, cool summers. The Canary Islands have subtropical climates that vary from hot desert to semi-arid, depending on altitude and orientation (Rubel and Kottek, 2010; Climate Shifts, 2025).

2.2. Ecological niche models for vectors

To obtain the ecological niche models for the vectors in the four study areas, the methodology described by Rodríguez-Escolar et al. (2023) and Morchón et al. (2023) was used. Of the four models, those corresponding to Madeira and the Azores Islands were developed for the first time, applying this methodological approach.

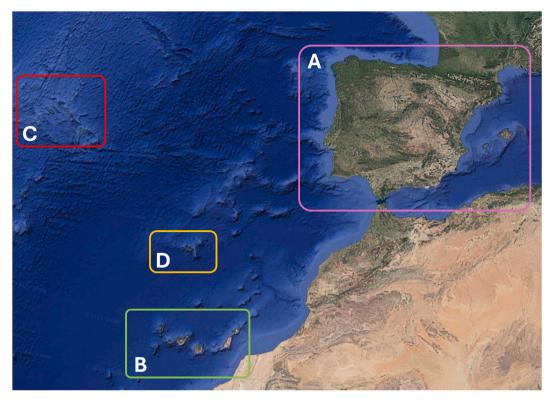


Fig. 1. Map of Portugal and Spain. A Iberian Peninsula and Balearic Islands. B Canary Islands. C Azores Islands. D Madeira.

2.2.1. Vector presence data and bioclimatic and environmental data

Published presence points for Cx. pipiens were used for Spain and Portugal, including the Iberian Peninsula, the Balearic Islands, the Azores Islands, Madeira, and the Canary Islands (Gomes et al., 2012; Osório et al., 2014; Ferreira et al., 2015; Gangoso et al., 2020). For the latter, data on the presence of the vector Cx. theileri were also used (Melero-Alcíbar et al., 2006, 2008; Morchón et al., 2011; Serafín-Pérez et al., 2022; Spanish Government, 2025). In addition, presence points from the GBIF database (http://www.gbif.org) were used to complete its distribution in the study areas (GBIF, 2024, 2025). The presence points were processed using QGIS software version 3.34.15 (QGIS Development Team, 2025). To this end, the data were spatially standardised by superimposing a grid with 1-km² cells, and just the centroid of each positive cell was considered for subsequent analysis. This was done to minimise possible biases associated with the spatial autocorrelation of the presence data. After processing the data, the final occurrence points were 1452 of Cx. pipiens in the Iberian Peninsula and Balearic Islands, 54 occurrence points of Cx. pipiens and Cx. theileri together in the Canary Islands, 8 occurrence points of Cx. pipiens in Madeira and 121 of Cx. pipiens in the Azores. In the case of the Canary Islands, both mosquito species were modelled as genus Culex, since very few points belonged to Cx. theileri and its distribution overlapped with that of Cx. pipiens.

The bioclimatic variables were taken from the World Clim database, which includes 19 variables related to temperature and precipitation between 1970 and 2000 (http://www.worldclim.org). These variables were selected based on a multicollinearity analysis in R software version 4.4.2 (R Core Team, 2024) using Pearson's correlation coefficient. Variables with a cross-correlation greater than or equal to 0.8 were excluded. The variables selected for the four study areas, taking into account the biology of the vector (Gangoso et al., 2020; Gorris et al., 2021; Rodríguez-Escolar et al., 2023; Morchón et al., 2023; Ragab et al., 2024), were: the annual mean temperature (°C) (BIO₁), isothermality (BIO₃), temperature seasonality (SD \times 100) (BIO₄), mean temperature of the wettest quarter (°C) (BIO₈), mean temperature of the driest quarter (°C) (BIO₉), annual precipitation (mm) (BIO₁₂), and precipitation

seasonality (coefficient of variation) (BIO₁₅). Other variables included are shrub and herbaceous density, from the EarthEnv database (http://www.earthenv.org/landcover); human footprint, obtained from SEDAC (SEDAC, 2025); and irrigated crops, rivers and water bodies (artificial and natural), downloaded from CORINE Land Cover (2025). All variables were downloaded and processed at a resolution of 1 km² with the same extent (study area) and the same coordinates (GCS_WGS_1984) using QGIS.

2.2.2. Habitat suitability models for vectors

To determine the potential distribution of vectors, four independent ENMs were developed: one for the Iberian Peninsula and the Balearic Islands, another for the Canary Islands, a third for Madeira, and a fourth for the Azores Islands. These models were generated using the MaxEnt (https://biodiversityinformatics.amnh.org/open_source/ maxent/), a predictive modelling tool based on the principle of maximum entropy that estimates the potential distribution of species using data on their presence (Phillips et al., 2006). To select the best-fitting model, the kuenm package of the R software was used, which generated and identified the best MaxEnt models from a set of candidates generated using different combinations of parameters (Cobos et al., 2019). A total of 102 candidate models were generated in each of the four cases, resulting from different combinations of different regularisation multiplier values to allow for different levels of 'localized' -'diffused' prediction (17 combinations, from 0.1 to 0.9 and from 1 to 10), a set of variables, and six combinations of feature classes representing the relationship between the species' presence and environment, linear (l), quadratic (q), and product (p) and combinations (l, q, lq, lp, qp and lqp). From these candidate models, kuenm selects the best fit based on three selection criteria: statistical significance (Partial_ROC < 0.05); omission rate (OR = 5%); and model complexity using the Akaike information criterion corrected for a small sample size (AICc < 2). In turn, the area under the curve (AUC) was assessed, which allows the overall predictive capacity of the models to be evaluated, with values close to 1 indicating high performance. The AUC values were calculated internally

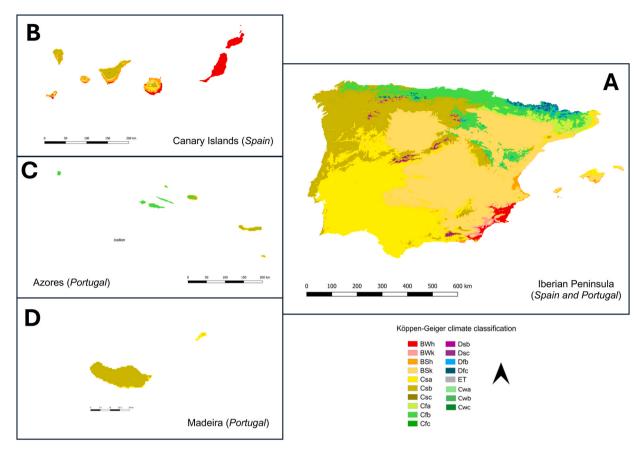


Fig. 2. Climates of Portugal and Spain according to the Köppen Climate Classification System: A Iberian Peninsula and Balearic Islands. B Canary Islands. C Azores Islands. D Madeira. *Abbreviations*: BSh, hot semi-arid climate; BSk, cold semi-arid climate; BWh, hot desert climate; BWk, cold desert climate; Cfa, humid subtropical climate; Cfb, temperate oceanic climate; Csa, hot-summer Mediterranean climate; Csb, warm-summer Mediterranean climate; Dfb, humid continental climate; Dfc, subarctic climate; Dsb, humid continental climate; Dsc, subarctic climate.

by *kuenm* using the standard MaxEnt procedure, which is based exclusively on presence records and 10,000 random background (pseudo-absence) points within the study area (default setting). These candidate models were constructed, evaluated and based on them, the final model was selected. The best-performing models therefore fulfilled all these criteria simultaneously, including statistical significance, low omission rate, reduced complexity, and high AUC values.

2.3. Generations of Dirofilaria immitis and risk maps by month

The next step was to obtain a monthly variable related to the development of the parasite within the vector, in order to combine it with the habitat suitability models and finally obtain infection risk maps for each month of the year. To do this, we calculated the number of generations of D. immitis in the vector (extrinsic incubation) according to the method described by Genchi et al. (2005) and Rodríguez-Escolar et al. (2023) using a custom script in R software, which calculates just the number of annual generations. This script has been modified to allow us to calculate and print the data on a monthly basis based on average daily temperatures, in addition to the annual data in a loop. For D. immitis to develop fully in the mosquito vector, 130 growing degree days (GDDs) must accumulate over a period of 30 consecutive days, which is the maximum life expectancy of the vector. It is also necessary for the average daily temperature to be at least 14 $^{\circ}\text{C}.$ To calculate these GDDs, the minimum required temperature of 14 $^{\circ}\text{C}$ is subtracted from the average daily temperature. Data on average daily temperatures from 1990 to 2016 were obtained from the CHELSA database (CHELSA Climate, 2025).

To create the risk maps, a weighting method was applied consisting

of multiplying, pixel by pixel, the estimated number of monthly generations of *D. immitis* with the vector habitat suitability models, using the QGIS raster calculator. The resulting risk maps showed a 50% contribution from the vector's ENM and a 50% contribution from the number of generations variable. Following this approach, general patterns of transmission risk through 30 years of climatic records were uncovered, which, unlike the real-time transmission tracker approaches (Atkinson et al., 2024a, 2024b) perform estimations based on recent historical data.

The values of the risk maps were established based on the monthly results obtained for each modelled territory. The maximum value corresponds to the month with the highest risk (high), the minimum to the month with the lowest risk (low), and the intermediate value was defined as the midpoint between them (medium). The labels were assigned in QGIS to facilitate visualisation and interpretation.

The annual risk maps for the Iberian Peninsula and the Canary Islands had already been validated in the studies of Rodríguez-Escolar et al. (2023) and Morchón et al. (2023) through correlation analyses between disease prevalence and estimated infection risk, as well as by using georeferenced data from more than 700 infected dogs. These validations demonstrated strong agreement and reliable predictive performance.

3. Results

3.1. Ecological niche models

The model selected for the vector corresponding to the Iberian Peninsula and the Balearic Islands obtained an AUC of 0.814, with a minimum suitability of 0.002 and a maximum of 0.85 (Supplementary Fig. S1). In the case of the Canary Islands, the model obtained an AUC of 0.730, with values ranging from 0.045 to 0.75. For the Azores Islands, the model presented the best fit of all, with an AUC of 0.908 and a minimum suitability of 0.0004 and a maximum of 0.92. Finally, the model for Madeira obtained an AUC of 0.805, with suitability values ranging from 0.00001 to 0.73.

The variable with the highest percentage contribution in the models was human footprint, except in the case of Madeira, where the variable that best explained the distribution of the species was the average temperature of the driest quarter. In the model for the Iberian Peninsula, the second variable with the highest contribution was the average temperature of the wettest quarter, while in the model for the Canary Islands, it was shrub density. In the case of Madeira, the second most relevant variable was the herbaceous density, and in the Azores Islands, it was the isothermality (Tables 1–4).

3.2. Number of generations of Dirofilaria immitis by month

The monthly evolution of the number of *D. immitis* generations in the Iberian Peninsula and the Balearic Islands is shown in Fig. 3. The areas with the highest annual number of generations were located on the Mediterranean coast, the south-west of the peninsula and the Balearic Islands, with a maximum of seven in July. During the winter, activity is reduced to two or three generations, limited to the south and the Mediterranean strip. In the Canary Islands (Fig. 4), coastal areas had more than four generations per year, while inland and higher-altitude regions, such as Teide, dropped to two or even one. The maximum was reached in July (almost six generations), and in winter, it remained in coastal areas, with an average of four generations. In the Azores Islands (Fig. 5), the number of generations ranged from zero to five, with a maximum in August and a minimum in February. Coastal and more populated areas showed greater activity, while higher areas, such as the island of Pico, showed the lowest values. Finally, in Madeira (Fig. 6), the maximum annual average was four generations, concentrated in coastal areas and Porto Santo. In the mountainous interior, activity decreased to one generation. The maximum was reached in August (up to five generations) and the minimum in January, when some activity was maintained only in coastal areas.

3.3. Potential risk of infection with Dirofilaria immitis by month

The risk of infection with *D. immitis* in the Iberian Peninsula and the Balearic Islands (Fig. 7) ranged from a minimum value of 0.6, referred to as "low" in the legend, to a maximum of 3.4, referred to as "high" in the legend, while "medium" corresponds to 2, considered a moderate risk. The risk is moderate to high in large areas of the territory, especially where favourable environmental conditions for the vector, *Cx. pipiens*,

Table 1Analysis of the contribution of environmental and bioclimatic variables to the ecological niche model for *Culex pipiens* in the Iberian Peninsula and the Balearic Islands (Spain).

Variable	Contribution (%)
Human footprint	57.8
Mean temperature of the wettest quarter (BIO ₈)	13.9
Temperature seasonality (BIO ₄)	4.1
Annual mean temperature (BIO ₁)	4.0
Precipitation seasonality (BIO ₁₅)	3.7
Mean temperature of the driest quarter (BIO ₉)	3.7
Rivers	3.0
Waterbodies	2.7
Herbaceus density	2.0
Isothermality (BIO ₃)	2.0
Annual precipitation (BIO ₁₂)	1.9
Shrub density	1.6
Irrigated crops	0.6

Table 2Analysis of the contribution of environmental and bioclimatic variables to the ecological niche model for *Culex pipiens* and *Culex theileri* in the Canary Islands (Spain).

Variable	Contribution (%)
Human footprint	82.2
Shrub density	14.1
Artificial waterbodies	2.0
Temperature seasonality (BIO ₄)	1.4
Herbaceus density	0.1
Mean temperature of the wettest quarter (BIO ₈)	0.1
Isothermality (BIO ₃)	0
Annual precipitation (BIO ₁₂)	0
Waterbodies	0
Precipitation seasonality (BIO ₁₅)	0
Irrigated crops	0
Mean temperature of the driest quarter (BIO ₉)	0
Annual mean temperature (BIO ₁)	0

Table 3Analysis of the contribution of environmental and bioclimatic variables to the ecological niche model for *Culex pipiens* in the Azores Islands (Portugal).

Variable	Contribution (%)
Human footprint	79.6
Isothermality (BIO ₃)	6.3
Mean temperature of the wettest quarter (BIO ₈)	2.0
Rivers	1.9
Waterbodies	1.9
Temperature seasonality (BIO ₄)	1.8
Mean temperature of the driest quarter (BIO ₉)	1.5
Annual mean temperature (BIO ₁)	1.4
Herbaceous density	1.3
Precipitation seasonality (BIO ₁₅)	1.0
Shrub density	0.6
Annual precipitation (BIO ₁₂)	0.5
Irrigated crops	0

Table 4Analysis of the contribution of environmental and bioclimatic variables to the ecological niche model for *Culex pipiens* in Madeira (Portugal).

Variable	Contribution (%)
Mean temperature of the driest quarter (BIO ₉)	34.3
Herbaceous density	30.9
Annual mean temperature (BIO ₁)	20.4
Human footprint	9.5
Isothermality	2.7
Precipitation seasonality (BIO ₁₅)	1.7
Mean temperature of the wettest quarter (BIO ₈)	0.3
Annual precipitation (BIO ₁₂)	0.2
Irrigated crops	0
Waterbodies	0
Temperature seasonality (BIO ₄)	0
Shrub density	0

coincide with a high number of annual generations of the parasite. The highest risk was found on the Mediterranean coast and in the south and centre of the peninsula, while areas at higher altitudes and with lower temperatures presented a low risk. The monthly development of the risk (Fig. 7) showed a large increase during the summer months, with peaks in July and August, and a decrease in winter, when the risk practically disappeared throughout the territory. However, in the south of the peninsula, the Mediterranean coast and the Balearic Islands, a certain level of risk persisted, albeit very low, even during the coldest months.

In the Canary Islands (Fig. 8), the risk ranged from 1 (low) to 3.1 (high), with 2 (medium) being a moderate value. The risk also peaked in summer, although it remained moderate during winter, suggesting a potential risk throughout the year. Coastal and low-altitude areas, with higher humidity and population density, presented the highest risk,

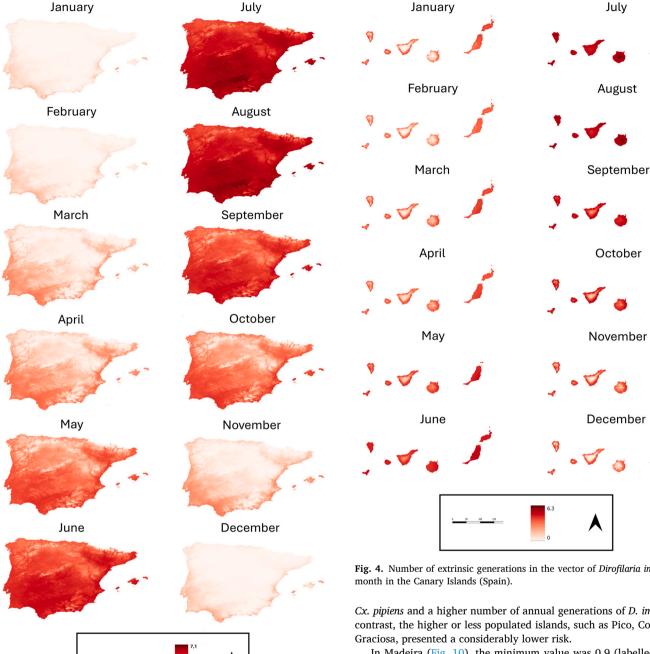


Fig. 3. Number of extrinsic generations in the vector of Dirofilaria immitis per month in the Iberian Peninsula (Portugal and Spain) and the Balearic Islands (Spain).

associated with high suitability for Cx. pipiens and Cx. theileri, and favourable conditions for a large number of generations of the parasite. In contrast, mountainous and high-altitude areas, such as Teide in Tenerife or the centre of La Palma, showed a low risk.

In the Azores Islands (Fig. 9), the ranges were from 1.3, referred to as "low" in the legend, to 3, referred to as "high", and a moderate value of 2.1, being the "medium" value in the legend. The maximum risk is recorded in August and the minimum in February. The most affected areas are low-lying and densely populated areas, such as Ponta Delgada in São Miguel, the south and east coasts of Terceira, and the city of Horta. These regions combine ideal conditions for the development of

Fig. 4. Number of extrinsic generations in the vector of Dirofilaria immitis per

Cx. pipiens and a higher number of annual generations of D. immitis. In contrast, the higher or less populated islands, such as Pico, Corvo, and

In Madeira (Fig. 10), the minimum value was 0.9 (labelled "low") and the maximum was 3 (labelled "high" in the legend), with 1.9 being a moderate value (labelled "medium"). The seasonal pattern was similar, with maximum risk in August and minimum risk in January. Coastal areas, especially Funchal and the Island of Porto Santo, were at risk throughout the year due to their low altitude and favourable conditions for the vector. Mountainous inland areas, such as Pico Ruivo, showed the lowest risk, with a clear altitudinal gradient.

4. Discussion

This study expands our knowledge about the transmission of canine heartworm disease in Spain and Portugal by creating infection risk maps with high spatial and temporal resolution. To this end, in addition to the ENM of its vectors restricted to these study areas, the monthly seasonal variation in the number of generations of the parasite is incorporated, obtaining infection risk maps for each of the twelve months of the year, from January to December.

Previous studies developed predictive models for D. immitis in

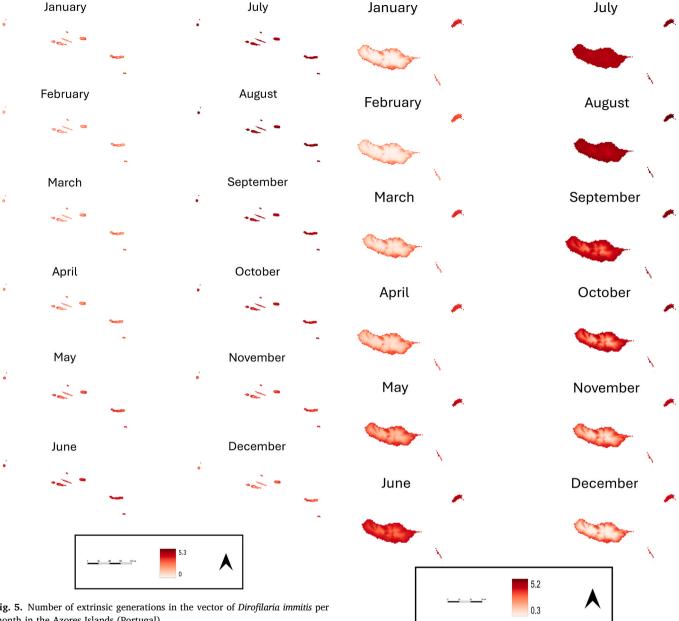


Fig. 5. Number of extrinsic generations in the vector of Dirofilaria immitis per month in the Azores Islands (Portugal).

Europe, at the continental and national levels, using GIS based on temperature records to estimate the number of generations of the parasite within the vector (Genchi et al., 2005, 2009, 2011; Rinaldi et al., 2006; Medlock et al., 2007; Mortarino et al., 2008; Kartashev et al., 2014; Ciuca et al., 2016). These studies assumed an oceanic climate with sufficient humidity for the vector to develop throughout western Europe, but did not take into account regions with a more arid or subtropical climate, nor the ideal habitat for vectors. On the other hand, studies have now been carried out in different European countries using ENMs of the vector and weighting them with the number of generations of the parasite to generate risk maps that are closer to reality (Morchón et al., 2023; Rodríguez-Escolar et al., 2023, 2024a, 2024b; Genchi et al., 2024). However, these models do not show the evolution of the risk of infection month by month throughout the year, as is the case for the Iberian Peninsula, the Balearic Islands, and the Canary Islands. Specifically, for Madeira and the Azores Islands, this process had not been carried out. Given this background, our objective was to develop risk models for Spain and Portugal and their island and peninsular territories for each month of the year in order to represent the

Fig. 6. Number of extrinsic generations in the vector of Dirofilaria immitis per month in Madeira (Portugal).

monthly progression of the infection. This approach will provide us with a dynamic view of the risk of infection with D. immitis and will allow us to identify critical months throughout the year.

The variable that showed the highest percentage contribution in the vector's ENMs was human footprint in all territories, except for Madeira, where it was BIO1 (average annual temperature). This suggests that areas with high human pressure provide an ideal habitat for the presence and establishment of the vector, as has also been proposed in other studies (Morchón et al., 2023; Rodríguez-Escolar et al., 2023, 2024a; Genchi et al., 2024). Large cities, together with high tourist pressure, make these areas an ideal habitat for mosquito vectors (Morchón et al., 2022). On the other hand, temperature-related variables, such as BIO₁, which had the highest percentage contribution in Madeira's ENM, have a positive influence on vector dispersal. This is because an increase in average temperature favours mosquito development and reduces the incubation time of larvae (Morchón et al., 2022).

July

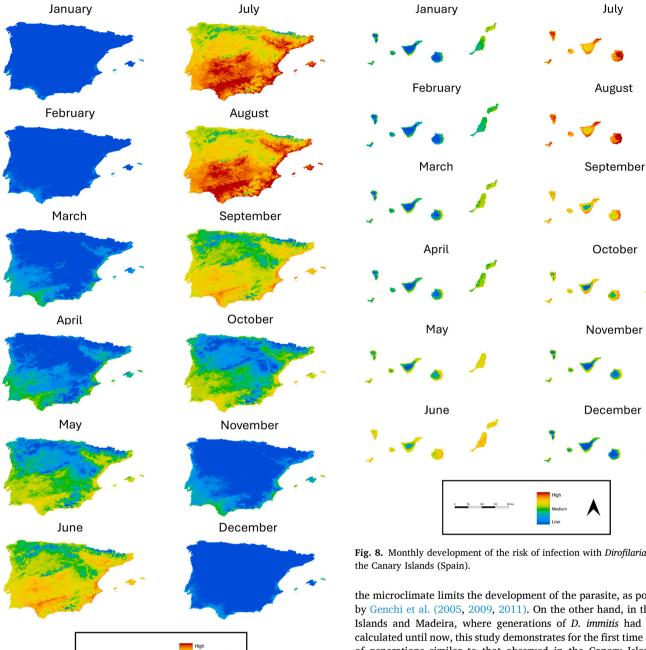


Fig. 7. Monthly development of the risk of infection with Dirofilaria immitis in the Iberian Peninsula (Portugal and Spain) and the Balearic Islands (Spain).

Regarding the number of generations of D. immitis, the results showed marked seasonality, with peaks during the summer and lows in winter, coinciding with the studies by Genchi et al. (2005, 2009, 2011), who linked transmission in Europe to ambient temperature. Even in the southern hemisphere, heartworm transmission has shown peak transmission rates during summer months (January and February) (Vezzani and Carbajo, 2006). The maxima of up to seven generations in the Iberian Peninsula correspond to their estimates for Mediterranean territories. The reduction in generations in winter and in higher altitude areas is also an aspect highlighted by Genchi et al. (2005). In the Canary Islands, the number of generations remained relatively constant throughout the year due to the thermal stability of the archipelago, although activity decreased in winter and in mountainous areas where

Fig. 8. Monthly development of the risk of infection with Dirofilaria immitis in

the microclimate limits the development of the parasite, as pointed out by Genchi et al. (2005, 2009, 2011). On the other hand, in the Azores Islands and Madeira, where generations of D. immitis had not been calculated until now, this study demonstrates for the first time a number of generations similar to that observed in the Canary Islands, with relatively stable levels throughout the year and a reduction in winter and in higher altitude areas.

The periods of greatest risk in the four models correspond to the summer months, where control campaigns should focus their efforts to reduce the incidence of the disease developed during later times in the year. This risk decreased progressively during autumn until it reached zero levels in most territories during the winter months. The areas of the peninsula with the highest risk are located in the south and on the Mediterranean coast, as well as in the Balearic Islands, where the risk remained very high even in the coldest months. In the Canary Islands, Azores Islands and Madeira, where a high risk was observed in the summer months, the risk, although low, also persisted in the winter months, concentrated in low-lying, densely populated areas, decreasing significantly in mountainous areas and regions with lower population density.

One of the limitations of this study was the lack of monthly data for some of the variables, which made it impossible to apply this approach to develop ENMs of the vector for each month of the year. This future

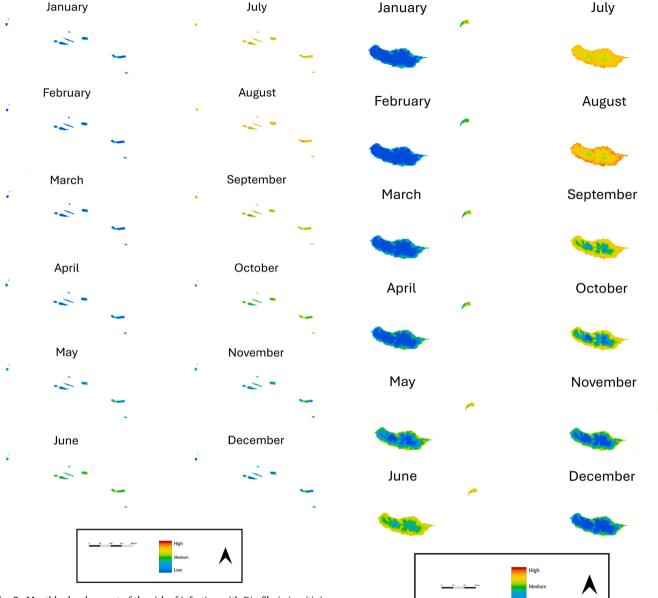


Fig. 9. Monthly development of the risk of infection with *Dirofilaria immitis* in the Azores Islands (Portugal).

line of research would allow for a more accurate representation of reality. For future studies, it would be beneficial to carry out ENMs on a monthly basis in order to identify more precisely the specific periods of the year when the establishment of the vector is most favourable.

Regarding the validation of the monthly risk maps, it is not currently possible to carry this out due to the lack of monthly data on infected dogs and the difficulty of obtaining such data reliably. Nevertheless, a future step of this project will focus on conducting such validation, which will require a large dataset of samples to ensure sufficient statistical robustness. This validation process is foreseen as part of a subsequent phase of the study.

Another limitation of the present study is the exclusive use of *Cx. pipiens* as the main vector, in addition to *Cx. theileri* in the case of the Canary Islands. In Spain and Portugal, several species of mosquitoes have also been reported in the peninsular region (*Anopheles maculipennis* (s.l.), *An. atroparvus*, *Aedes caspius*, *Ae. detritus* (s.l.), and *Cx. quinque-fasciatus*). There are also other potential vectors in which the presence of *D. immitis* has not yet been reported in these countries, but have recently been introduced into other European countries, such as *Ae. albopictus* and *Ae. vexans* (Goiri et al., 2020; Varga et al., 2025). A future line of

Fig. 10. Monthly development of the risk of infection with *Dirofilaria immitis* in Madeira (Portugal).

work would be the inclusion of other species that act as vectors in the territory, which would allow for a more complete prediction of the risk of infection.

Finally, this tool is primarily intended for pet owners and veterinary professionals, providing guidance to reinforce prevention and vector control measures during the months of highest transmission risk. Developed within a One Health framework, it underscores the importance of dirofilariosis from a public health perspective, highlighting when and where preventive measures should be implemented, continuously in some areas, and only during specific months in others.

5. Conclusions

Due to the continuous spread of diseases with potential zoonotic impact, which is exacerbated by anthropogenic climate change, among other factors, it is essential to conduct studies to determine which environmental circumstances favour this spread. Predictive modelling of

vector-borne diseases requires the incorporation of key variables (such as vector habitat suitability, parasite behaviour within the vector, and human footprint) that influence disease dynamics in order to generate spatially detailed risk models with high predictive power. Our models showed that in Spain and Portugal, the risk of infection with D. immitis persists throughout the vear in the Iberian Peninsula, the Balearic Islands, the Canary Islands, the Azores Islands and Madeira. The areas identified as being at greatest risk of infection include the coastal regions of the south and east of the peninsula, the Balearic Islands and most of the Canary Islands, the Azores Islands and Madeira, with the risk decreasing in higher altitude areas. This risk increases in the summer months, especially July and August, while it decreases during the winter months, so measures to control the incidence of infection should be implemented according to the time of year. These results allow us to anticipate epidemiological scenarios and thus guide surveillance, prevention and control strategies more effectively, adjusting them to the specific conditions of each territory throughout the year. Even so, it is necessary to continue studies at the local level that delve deeper into the dynamics of transmission and make it possible to prevent the spread of the disease under changing environmental conditions.

Ethical approval

Not applicable.

CRediT authorship contribution statement

Elena Infante González-Mohino: Writing – original draft, Writing – review & editing, Resources, Investigation, Formal analysis, Data curation. Iván Rodríguez-Escolar: Writing – original draft, Writing – review & editing, Methodology, Validation, Supervision, Data curation. Alfonso Balmori-de la Puente: Writing – original draft, Writing – review & editing, Validation, Supervision. Manuel Collado-Cuadrado: Writing – review & editing, Investigation, Formal analysis. Elena Carretón: Writing–review & editing, Investigation. José Alberto Montoya-Alonso: Writing–review & editing, Investigation. Rodrigo Morchón: Writing – original draft, Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Supervision, Funding acquisition, Conceptualization.

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Declaration of competing interests

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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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2025.100330.

Data availability

The data supporting the conclusions of this article are included within the article. Raw data will be made available on request.

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