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The historical ecological background of West Nile virus in Portugal indicates One Health opportunities

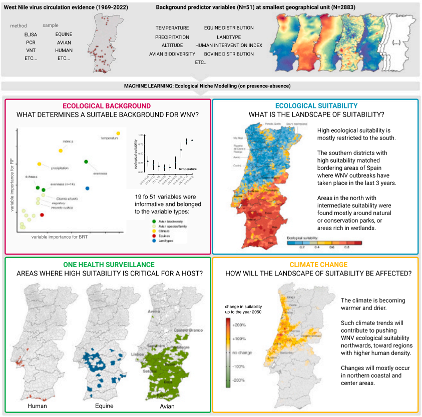
Martim A. Geraldes[a,b], Mónica V. Cunha[a,b], Carlos Godinho[c], Ricardo F. de Lima[a,d], Marta Giovanetti[e,f,g,h], José Lourenço[b,i,j,*]

[a] *Centre for Ecology, Evolution and Environmental Changes (cE3c), CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal*
[b] *Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal*
[c] *MED - Mediterranean Institute for Agriculture, Environment and Development, LabOr - Laboratory of Ornithology, Instituto de Investigação e Formação Avançada, Universidade de Évora, Évora, Portugal*
[d] *Centro de Biodiversidade do Golfo da Guiné (CBGG), São Tomé, São Tomé and Príncipe*
[e] *Laboratório de Flavivírus, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil*
[f] *Instituto Rene Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil*
[g] *Department of Science and Technology for Humans and the Environment, Università of Campus Bio-Medico di Roma, Italy*
[h] *Climate amplified diseases and epidemics (CLIMADE) Americas, Brazil*
[i] *Universidade Católica Portuguesa, Católica Medical School, Católica Biomedical Research Centre, Portugal*
[j] *Climate amplified diseases and epidemics (CLIMADE) Europe, Portugal*

HIGHLIGHTS

- West Nile virus is a zoonotic virus that is expanding geographically and increasing in epidemic activity across European countries
- Portugal is an outlier among Mediterranean countries by not having experienced a large human-associated outbreak yet
- Ecological backgrounds suitable for WNV circulation are located to the south, identified to have warmer and drier climates, high avian diversity, presence of specific avian species and land types
- Climate change will slowly expand the range of ecological backgrounds suitable for WNV circulation northwards
- A number of identified localities are critical for WNV future surveillance in Portugal

GRAPHICAL ABSTRACT



19 fo 51 variables were informative and belonged to the variable types:

High ecological suitability is mostly restricted to the south.

The southern districts with high suitability matched bordering areas of Spain where WNV outbreaks have taken place in the last 3 years.

Areas in the north with intermediate suitability were found mostly around natural or conservation parks, or areas rich in wetlands.

The climate is becoming warmer and drier.

Such climate trends will contribute to pushing WNV ecological suitability northwards, toward regions with higher human density.

Changes will mostly occur in northern coastal and center areas.

Abbreviations: WNV, West Nile virus; RFE, Recursive Feature Elimination.; ROC, Receiver Operating Characteristic (ROC).; RF, random forest (RF).; BRT, boosted regression trees (BRT).; TNR, true negative rate (TNR).; TPR, true positive rate (TPR).; ML, machine learning.; DGAV, direção geral de alimentação e veterinária (national veterinary and phytosanitary health authority).

[*] Corresponding author at: Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.
E-mail address: jolourenco@ucp.pt (J. Lourenço).

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ABSTRACT

West Nile (WNV) is a zoonotic arbovirus with an expanding geographical range and epidemic activity in Europe. Not having yet experienced a human-associated epidemic, Portugal remains an outlier in the Mediterranean basin. In this study, we apply ecological niche modelling informed by WNV historical evidence and a multitude of environmental variables from across Portugal. We identify that ecological backgrounds compatible with WNV historical circulation are mostly restricted to the south, characterized by a warmer and drier climate, high avian diversity, specific avian species and land types. We estimate WNV ecological suitability across the country, identifying overlaps with the distributions of the three relevant hosts (humans, birds, equines) for public and animal health. From this, we propose a category-based spatial framework providing first of a kind valuable insights for WNV surveillance in Portugal under the One Health nexus. We forecast that near future climate trends alone will contribute to pushing adequate WNV ecological suitability northwards, towards regions with higher human density. This unique perspective on the past, present and future ecology of WNV addresses existing national knowledge gaps, enhances our understanding of the evolving emergence of WNV, and offers opportunities to prepare and respond to the first human-associated epidemic in Portugal.

1. Introduction

West Nile virus (WNV) is a mosquito-borne virus within the *Flaviviridae* family, first identified in 1937 in the West Nile district of Uganda. The ecology of the virus is characterized by a zoonotic transmission cycle maintained between mosquitoes and avian species. Six mosquito genera have been implicated in transmission, but *Culex* spp. (e.g. *Culex pipiens*, *Culex univitattus / perexiguus, Culex modestus, Culex tarsalis, Culex restuans*) are commonly referred to as the main vectors in Europe and North America. WNV is genetically diverse and currently up to nine lineages have been proposed (Fall et al., 2017). Occasional zoonotic viral spillover can occur from the avian-mosquito cycle into human populations, as well as to domesticated and wild mammals (Granwehr et al., 2004; Campbell et al., 2002; Petersen et al., 2013). To date, most human-associated outbreaks across the globe have been attributed to viral lineages 1, 2 and 5 (Chancey et al., 2015).

Among incidental mammal hosts that get infected through mosquito-bites, humans and equines feature predominantly in epidemiological data, with equines often serving as a sentinel species since outbreaks among equines often preclude human cases. Live and dead birds are also sometimes used as sentinels, in particular to monitor transmission levels via seroprevalence surveys (Lauriano et al., 2021) or viral detection (Tamba et al., 2024) under passive and active surveillance schemes. Reports of recent outbreaks among avian species or equines can be used to trigger local human prevention initiatives (Riccardo et al., 2020; Young et al., 2019). Contrary to most avian species, mammals are inefficient amplifier hosts, and hence cannot establish mosquito-mammal transmission cycles (Gamino and Höfle, 2013; Bunning et al., 2002; Petersen et al., 2013). Approximately 80 % of human infections are asymptomatic, whereas the rest may develop mild or severe neuroinvasive disease with an associated mortality risk (Hayes et al., 2005; McDonald, 2021). A mixture of co-factors related to health status (e.g. diabetes and hypertension) have been identified as risk factors for severe neuroinvasive disease (Lindsey et al., 2012)). There are currently no licensed vaccines nor antiviral treatments for humans (Petersen et al., 2013; Saiz, 2020), but four equine licensed vaccines are available (Saiz, 2020).

Introduction and increased epidemic activity of mosquito-borne viruses is being promoted by globalized trends related to climate and human activity (Rizzoli et al., 2019; Wang et al., 2020; Braack et al., 2018; Johnson et al., 2018; Gould et al., 2017; Erazo et al., 2024). Historically, epidemic activity of WNV was mostly reported from African countries and Israel (Murgue et al., 2002). At the turn of the century, awareness of WNV changed globally when it was introduced to New York city, from where it later became endemic across the USA, Canada, Mexico, Central and South America (Pybus et al., 2012; Kilpatrick et al., 2007). In the decade following introduction in the USA, WNV also seemingly became more active in Europe, Middle East and Russia (Johnson et al., 2018; Murgue et al., 2002). WNV epidemiology exhibits significant spatiotemporal heterogeneities within and between countries, the drivers of which are not entirely characterized. The most well described driver is local climate variation, with well demonstrated effects on mosquito and avian life-cycles and population dynamics, consequently influencing local WNV epidemic activity, dispersal and persistence (Dellicour et al., 2020; Shocket et al., 2020; Petersen et al., 2013; Erazo et al., 2024).

In Europe, recent increasing epidemic activity has been reported in France, Italy, Greece, Hungary, Romania, Bulgaria, Serbia and Ukraine (Haussig et al., 2018; Riccardo et al., 2020). The largest epidemic ever was recorded across the continent in 2018, with 2083 cases exceeding cumulative infections since 2010 (Bakonyi and Haussig, 2020). In 2020, Spain reported a WNV epidemic which unprecedentedly included a large number of human infections and deaths, including regions not previously known to be affected by WNV (ECDC, 2020; García San Miguel Rodríguez-Alarcón et al., 2021; Figuerola et al., 2022). In 2022, the second highest ever yearly number of locally acquired cases was reported in Europe with a total of 1133, and concurrently the number of local cases in Italy was the highest ever recorded (ECDC, 2023; Branda et al., 2023).

These recent trends, particularly in countries around the Mediterranean basin sharing ecological conditions, highlight Portugal as an outlier. Despite evidence of WNV circulation in the country since 1969 (Filipe and Pinto, 1969), Portugal has not experienced a human-associated epidemic. For reasons not well understood, only four serologically confirmed human infections have been reported to date (Lourenço et al., 2022; Mencattelli et al., 2023). Since 1996 laboratory diagnostics on humans and vectors have been managed by Instituto Nacional de Saúde Dr. Ricardo Jorge (National Health Institute Dr. Ricardo Jorge) and on non-human animals by Instituto Nacional de Investigação Agrária e Veterinária (National Agrarian and Veterinary Research Institute). Detection of WNV cases in mosquitoes, humans and other animals is mandatory in Portugal, but surveillance remains mostly reactive to the sporadic reports of symptomatic horses, often but not always resulting in localized sampling and testing of horse and mosquito populations (DGAV, n.d.). Since 2008 Portugal has performed active vector surveillance by the Rede de Vigilância de Vetores (Network of Vector Surveillance, REVIVE) for which *Flavivirus* testing (including WNV) is performed in mosquitoes, but with no positive cases identified so far (REVIVE, n.d.). It is possible that the rarity of human cases is partially explained by underreporting, driven by a combination of a low symptomatic rate (Hayes et al., 2005), lack of medical and veterinary awareness, and the current passive surveillance system. This possibility is exemplified by two of the four reported human cases that occurred in Irish birdwatchers that visited the bird reserve of Ria Formosa (Algarve), who became symptomatic near the end of their trip in Portugal, and were only detected after returning to Ireland (Connell et al., 2004).

Vaccination against WNV in equine populations is not mandatory in Portugal and reported vaccination numbers are low according to official data from the national veterinary and phytosanitary health authority (DGAV) (e.g. between 2016 and 2021 a total of 1608 equines were vaccinated across the country). It remains unclear if the virus circulates endemically or not, with a total of only two genome sequences generated to date being unable to support either scenario (Mencattelli et al., 2023). Previous accumulated evidence indicates that WNV is largely concentrated in the south where the climate is Warm Temperate Mediterranean, contrasting to that of the north which is Temperate Mediterranean (Köppen-Geiger classifications) (Beck et al., 2018; Lourenço et al., 2022).

There are many factors universally understood to modulate WNV epidemic activity that have not been explored within the Portuguese context (e.g. land types, host distributions, avian biodiversity). In the current study, we modelled and explored the ecological backgrounds and suitability for WNV transmission in Portugal. Our general aim was to elaborate on previous research, by applying modelling techniques to an expanded historical dataset (1969–2022) of past WNV evidence and a large number of animal and environmental variables across the country, allowing for the exploration of a myriad of possible WNV-related factors beyond the single contribution of local climate. Major goals included: (i) to explore the relationships of a number of data sources and types related to the key players in WNV surveillance and health (humans, equines, avian species), climate and landscape with historical WNV circulation thus generating insights into compatible ecological backgrounds with WNV transmission; (ii) identify avian species linked to historical WNV circulation; (iii) project the spatial likelihood of WNV circulation across the territory; (iv) develop the first category-based spatial framework providing first of a kind valuable insights for current and future WNV surveillance in Portugal under the One Health nexus; and (v) explore the possibility that future climate change may alter the likelihood landscape of WNV circulation across the territory. We identified that ecological backgrounds showing higher suitability for circulation (i.e. compatible with evidence) were mostly restricted to the south of the country, generally defined by warmer temperatures, lower precipitation, high avian diversity, unique land types and prevalence of specific avian species. We leverage the ecological background and suitability outputs to propose a novel suitability and evidence based framework. We characterize regional potentials for surveillance initiatives under a One Health perspective, including the three-host axes of WNV surveillance and public and animal health importance (humans, equines and birds). Finally, given the identified, predominant role of local climate in our analyses, we performed a projection exercise accounting for observed, long-term climate change trends to estimate the changing landscape of ecological suitability across the country. We found that climate change alone will contribute to an expansion of regions suitable for WNV circulation. Together, our results reiterate that the south of Portugal is ecologically suitable for WNV transmission, and support a change towards active surveillance in the coming years.

2. Material and methods

2.1. WNV occurrence data

The evidence data for this study was collected from multiple sources to comprehensively assess the presence and distribution of WNV occurrences in Portugal. Initially, an occurrence list from our previous literature review was considered (Lourenço et al., 2022). To complement this, a partnership with Direção Geral de Alimentação e Veterinária (DGAV), the national veterinary and phytosanitary health authority was established, to include historical WNV outbreak information that was not publicly available. A second literature review was conducted to supplement the existing data up to 2022. Additionally, a recent master thesis by Costa et al. (Costa, 2021) provided data on WNV serological testing of avian species. For analysis purposes, we summarized existing evidence into a binary classification of WNV "present" or "pseudo-absent" per the smallest administrative geographical unit in Portugal, termed freguesia, of which there are 2882 in total.

2.2. Predictive variables data

A total of 51 biotic and abiotic environmental variables (here termed predictor variables) per freguesia were considered for analysis. These included factors related to climate, mammal and avian species, mosquitoes, land types, landscape, avian biodiversity and human population. Detailed information on variable selection, sources and curation is included in Supplementary Text 1 and variable listing is included in Supplementary Table S2.

2.3. Ecological niche modelling

We applied two machine learning (ML) approaches informed by the collected WNV occurrence data and predictive variables data: boosted regression trees (BRT) and random forests (RF). The output of these models was used to unravel and explore WNV ecological backgrounds (observed relationship of predictive variables with likelihood of WNV presence status) and quantifying WNV ecological suitability per freguesia (estimated likelihood of WNV presence status). The estimated WNV ecological suitability per freguesia of each ML model was combined to inform a metalearner model (Generalized Linear Model with negative weights) for a final estimation of local WNV ecological suitability per freguesia. For ML modelling classification of WNV present and pseudo-absent, we considered a conservative threshold of 0.5 (similar to previous studies (Klitting et al., 2022; Amdouni et al., 2022)). When performing machine learning with BRT and RF approaches, a balanced training dataset is recommended in terms of having similar numbers of data entries per possible class for classification (in this case, present and pseudo-absent) (Kumari and Srivastava, 2017). A total of 59 freguesias presented past evidence for WNV circulation. We thus devised a subsampling strategy that we term m-folds (similar to k-folds in cross-validation) in which a total of m BRT and RF models are ran, each considering a subset of the data including training subsets composed of 80 freguesias randomly sampled with replacement, with guaranteed equal proportions of freguesias with WNV present and pseudo-absent status. The major benefit of this approach being that all model runs consider a balanced training dataset and that the smaller number of freguesias with WNV present status are compared and analyzed versus the larger number of freguesias with WNV pseudo-absent status. For modelling sensitivity and decision making, we considered up to 1000 m-folds. For some of the analyses (when explicitly mentioned), we determined an adequate, smaller than 1000 number of m-folds, by calculating and checking convergence of model performance (stopping at m-folds number above which more runs would not affect model performance). Results are presented as the mean output of the models across the m-folds runs, unless stated otherwise. A detailed description of the ML methods, parameterization and software packages used is included in Supplementary Text 1. All computational tasks were performed using R v4.2.1.

2.4. Variable selection

We applied recursive feature selection by removing variables based on their measured importance in the BRT and RF models. After removal of the least important variable, the performance metrics of the models were recalculated. The iterative removal of variables stopped once the most sensitive metric (identified in the results section as ROC from the BRT model) decreased by >1 % of its value across the m-folds when using all $N = 51$ variables.

3. Results

We collected evidence for WNV occurrence in mainland Portugal (1969–2022). Given the existing passive surveillance framework and the fact that a large portion of the reported evidence did not include geographic coordinates, we summarized existing evidence by attributing *present* or *pseudo-absent* status per freguesia (Fig. 1A). Historical evidence included samples from birds, equines, humans, other mammals and vectors (Fig. 1B). The predominant diagnostic methods were enzyme-linked immunosorbent assays (ELISA), viral neutralization testing (VNT), hemagglutination inhibition testing (HI), other serology-based methods, reverse transcriptase polymerase chain reaction (RT-PCR), and viral isolation (Fig. 1C).

Evidence of WNV circulation became more frequent post 2010, with a significant proportion (67 %) of reports from equines. The spatial distribution of positive evidence was mostly restricted to the south and seemingly did not follow a pattern similar to the distribution to the main WNV vectors (*Culex spp*) in Portugal according to national surveillance data (but similar to modelling estimates e.g. (Gangoso et al., 2020)) nor that of equines (both of which are essentially present across the entire latitudinal range of the country, Supplementary Fig. S1). Interestingly, some of the southern districts where evidence has accumulated share borders with the Spanish regions of Extremadura and Andalusia (Fig. 1A), where WNV has recently caused outbreaks involving multiple host species (García-Carrasco et al., 2023; García San Miguel Rodríguez-Alarcón et al., 2021).

3.1. Predictive variables

We collected 51 variables that potentially influence local WNV transmission (Supplementary Tables S2–3). These predictive variables served as input for ecological niche modelling towards explaining the historical geographical distribution of WNV evidence. For this, we designed an ensemble modelling approach supported by the output of two machine learning models: a boosted regression trees (BRT) and a random forest (RF) model. In essence, each machine learning model was informed by the predictive variables to classify the WNV evidence status of each of the 2882 freguesias (present, pseudo-absent) under a cross-validation subsampling strategy. The resulting classification from both models was then used as the predictive variables to inform an ensemble

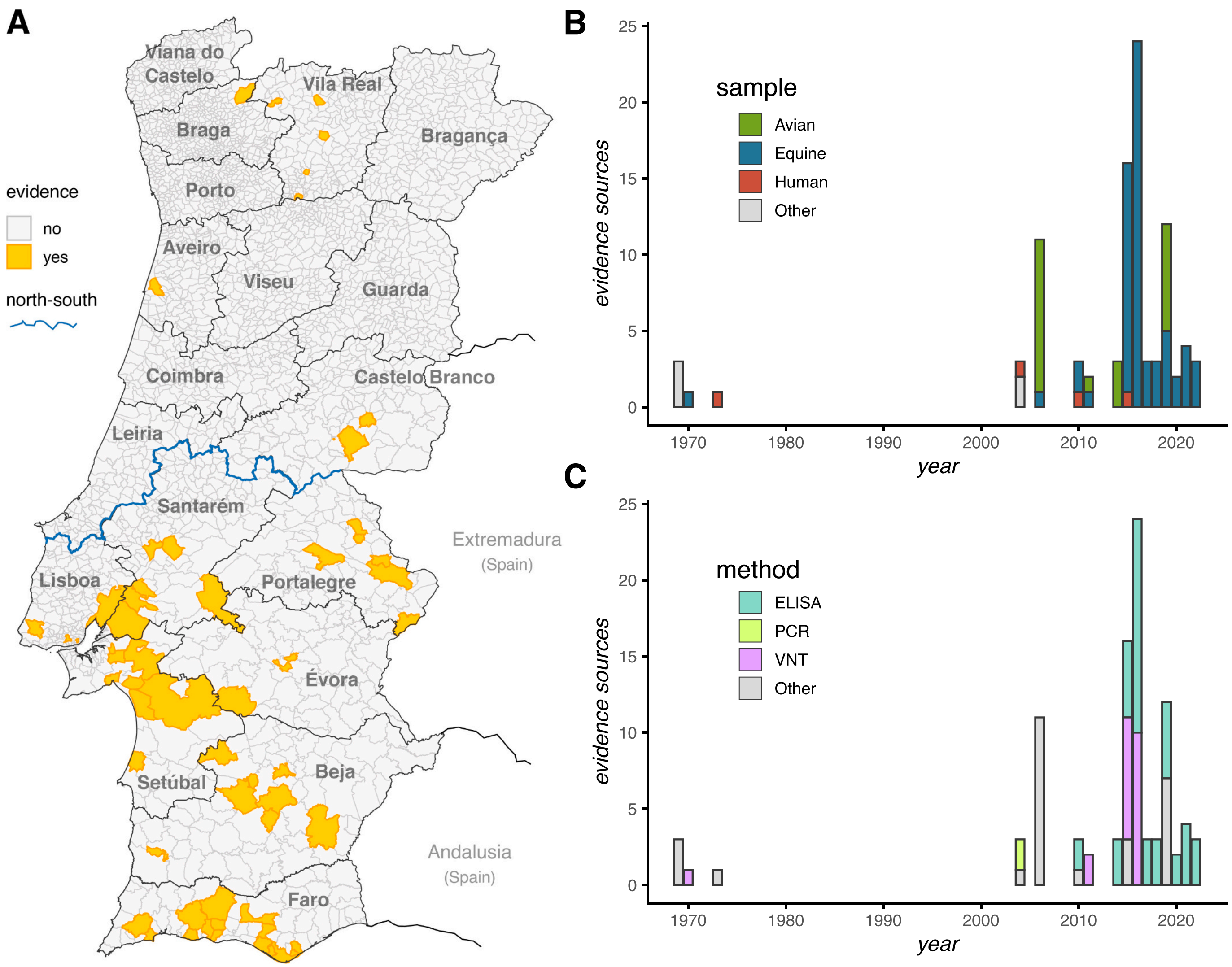


Fig. 1. WNV historical evidence in Portugal (1969–2022). (A) Map of Portugal, presenting the geographical distribution of historical evidence for WNV presence (yellow). Freguesias, the smallest geographical units ($N = 2882$) have grey borders, while districts ($N = 18$) have black borders and are named. The blue line marks the north-south geographical division. The regions of Extremadura and Andalusia of Spain are also highlighted bordering to the south of Portugal. Total number of evidence sources by (B) sampled host and (C) testing method by year (of publication). In (C), PCR stands for reverse transcriptase PCR. Full dataset available in Supplementary Table S1.

model on a final classification exercise.

Firstly, Recursive Feature Elimination (RFE) excluded the least informative variables while minimizing cost to model performance. In summary, we provided both BRT and RF models with a decreasing number of predictive variables, iteratively removing the variable with least measured importance. Performance of both models was generally stable while removing many variables, only sharply decreasing after removing about 40 of the 51 available variables (Fig. 2A). The performance metric Receiver Operating Characteristic (ROC) from the BRT model was the most sensitive, decreasing early on, after removal of 20 variables. Based on this sensitivity, we applied a conservative, heuristic approach that would stop RFE once the BRT ROC would fall by >1 % of its initial range (when using all 51 predictor variables). The BRT ROC fell below such initial performance range after removing the variable *Turdus merula* (Common Blackbird). The retained 19 variables after the RFE process are detailed in Table 1.

3.2. Ecological niche modelling

Using the 19 selected variables by RFE (Table 1), we measured model performance in classifying WNV evidence status per *freguesia* (Table 2). The true positive rate (TPR) was higher than the true negative rate (TNR), and overall accuracy appeared capped by the performance of the TNR. On one hand this is an expected result from the highly diverse ecological background of freguesias with status WNV pseudo-absent (covering the entire territory) compared to that of freguesias with status WNV present (concentrated in the south). As such, it was more likely for the models to misclassify freguesias with pseudo-absent status given their diverse ecological backgrounds. On the other hand, it indicated that some freguesias with pseudo-absent status had similar ecological backgrounds to those with present status (i.e. predicting possible circulation in areas without existing evidence, explored in the results at a later stage).

3.2.1. Ecological background

The ecological backgrounds suitable for WNV circulation could be explored through the knowledge emerging from selecting predictive variables, their relative importance for the underlying models, and their relationships with the estimated likelihood of WNV presence per freguesia.

The BRT and RF models generally agreed on the relative importance of the 19 predictive variables with small variation (Fig. 2B), likely linked to their capacity to best capture linear (BRT) versus more complex (RF) variable relationships. With highest importance were variables related to climate (temperature, index P, precipitation), landscape (land type) and avian biodiversity (evenness, calculated over all avian species in the eBird dataset). Other avian biodiversity metrics were also selected but with less importance, such as biodiversity measured via richness over all species (variable *richness*), evenness over 14 hand-curated species (variable *evenness* $n = 14$) and prevalence of migratory species (variable *migratory*). The distributions of nine avian species were also deemed important, with particular emphasis on *Ciconia ciconia* (White Stork) and *Hirundo rustica* (Barn Swallow). Such specific species had less importance than the climatic, landscape and most of the avian biodiversity measures. Finally, the distribution of equines was also selected but ranked low in importance compared to most other variables for both models.

Suitable ecological backgrounds were found to be associated with specific land types, including wetlands, shores (e.g. dunes), salt marshes, leisure facilities (e.g. golf courts), or agro-forest fields and estuaries. Levels of ecological suitability were also positively associated with temperature~~s~~ above 15° Celsius and avian biodiversity, and negatively associated with precipitation. For specific avian species or groups of species, suitable backgrounds were found to be dependent on either a monotonic relationship with a species' or group's abundance (e.g. *Erithacus rubecula, Columba palumbus, Garrulus glandarius,* Corvids), or simply dependent on the presence of a species or group (e.g. *Ciconia ciconia, Anas platyrhynchos, Hirundo rustica,* migratory).

Given the nature of the modelling approach, the described relationships between input variables and suitable ecological backgrounds remain indicative of associations with WNV circulation but did not discern a causative effect. A full description on the relationships and contributions of all 19 variables to suitable ecological backgrounds across the freguesias is included in Supplementary Text 1.

3.2.2. Ecological suitability

According to the ensemble model ecological suitability was highly variable across the country (Fig. 3A, with the independent output of the BRT and RF models presented in Supplementary Fig. S3). Highest suitability was estimated across the southern part of the country, namely

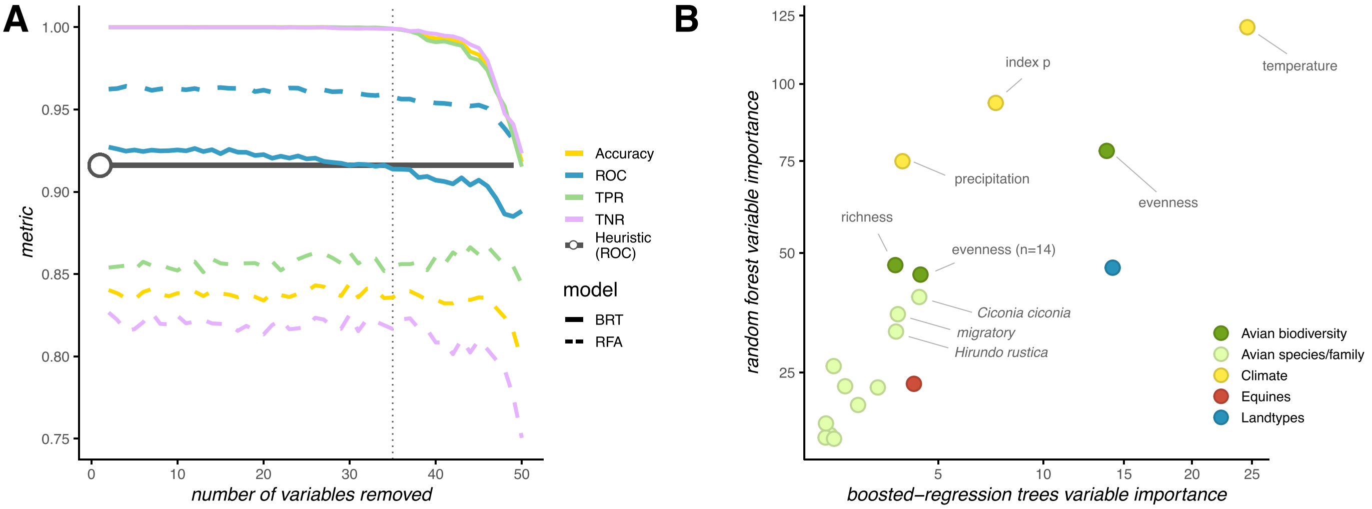


Fig. 2. Metrics on recursive feature elimination and final variable importance. (A) Metrics of both the BRT (booster-regression trees, full lines) and RF (random forest, dashed lines) models when iteratively removing variables, one by one (ROC = Receiver Operating Characteristic; Accuracy = proportion of correct classifications; TPR = true positive rate or sensitivity defined as evidence status "present"; TNR = true negative rate or specificity defined as evidence status "pseudo-absent"). The dark grey line and point highlight the heuristic value used to stop the recursive feature elimination (1 % decrease of the minimum of ROC BRT model, across the m-folds, when using all 51 variables). (B) Variable importance for both models using the final variables not excluded by the recursive feature elimination ($N = 19$). Variables are aggregated into types (as in legend) for visualization and detailed in Table 1.

Table 1
Final set of 19 predictive variables from the Recursive Feature Elimination (RFE) approach. Sources, types and curation of variables detailed in Supplementary Table S2. * Relative abundance to all other avian species. ♪ See Supplementary Text 1. Ebird refers to the platform ("eBird: A Citizen-Based Bird Observation Network in the Biological Sciences", 2009). C.N. = "Common name". S.N.: = "Scientific name".

Variable	Description	Notes	Source
Turdus merula	Abundance *	C.N.: Common Blackbird; S.N.:*Turdus merula*	eBird
Garrulus glandarius	Abundance *	C.N.: Eurasian Jay; S.N.: *Garrulus glandarius*	eBird
Hirundo rustica	Abundance *	C.N.: Barn Swallow; S.N.: *Hirundo rustica*	eBird
Erithacus rubecula	Abundance *	C.N.: European Robin; S.N.: *Erithacus rubecula*	eBird
Anas platyrhyncos	Abundance *	C.N.: Mallard S.N.: *Anas platyrhyncos*	eBird
Pica pica	Abundance *	C.N.: Eurasian Magpie S.N.: *Pica pica*	eBird
Columba palumbus	Abundance *	C.N.: Wood Pigeon S.N.: *Columba palumbus*	eBird
Ciconia ciconia	Abundance *	C.N.: White Stork S.N.: *Ciconia ciconia*	eBird
Cuculus canorus	Abundance *	C.N.: Common Cuckoo S.N.: *Cuculus canorus*	eBird
Corvids	Abundance *	C.N.: Carrion Crow, Common Raven, Eurasian Magpie, Eurasian Jay S.N.: *Corvus corone, Corvus corax, Pica pica, Garrulus glandarius*	eBird
Migratory	Abundance *	C.N.: White stork, Barn Swallow S.N.: *Ciconia ciconia, Hirundo rustica*	eBird
Evenness (n = 14)	Measure of abundance balance	14 species WNV-associated ♪	eBird
Evenness	Measure of abundance balance	All avian species	eBird
Equine	Abundance	Absolute numbers	INE
Richness	Number of species	All avian species	eBird
Index P	Theoretical transmission potential	Climate-based	(Lourenço et al., 2022)
Temperature	Temperature in Celsius	Mean Celsius	Copernicus.eu
Precipitation	Precipitation in mm	Mean mm	Copernicus.eu
Land types	Most common land type	As classified in dataset	Copernicus.eu

Table 2
Final model performance using the selected 19 predictive variables. Results presented are for 200 m-folds. Sensitivity of each model's performance for varying numbers of m-folds are presented in Supplementary Fig. S2. ROC = Receiver Operating Characteristic; Accuracy = proportion of correct classifications; TPR = true positive rate (sensitivity) defined as evidence status WNV *present*. TNR = true negative rate (specificity) defined as evidence status WNV *pseudo-absent*.

Model	ROC	Accuracy	TPR	TNR
Random forest (RF)	0.95	0.82	0.95	0.82
Boosted-regression trees (BRT)	0.94	0.81	0.93	0.81
Ensemble (EN)	0.95	0.82	0.94	0.82

across vast areas of the districts of Setúbal, Santarém, Portalegre, Évora, Beja and Faro, as well as more locally within the districts of Lisboa and Castelo Branco. The boundary of lowest to highest suitability between the north and the south colocalized with the separation of the two climate types present in the country (Temperate Mediterranean to the north, Warm Temperate Mediterranean to the south, according to Köppen-Geiger classification (Beck et al., 2018)). The identified southern region with highest suitability was also in accordance with a recent study estimating risk of WNV transmission to humans and equines across the Iberian Peninsula using generalized linear modelling and a partially overlapping but different set of predictive variables (Supplementary Fig. S4) (García-Carrasco et al., 2023). Notably, some smaller areas in the north were also estimated to harbour intermediate-to-high ecological suitability, namely around the urban centers of Aveiro, Vila Real and Covilhã, as well as the national park of Peneda-Gêres and the natural parks of Figueira de Castelo Rodrigo and Douro Internacional (Fig. 3A). All estimated suitability values per freguesia are made available in Supplementary Table S5.

3.3. Towards a One Health suitability and evidence based framework

Estimated ecological suitability across the country showed that the vast majority of freguesias had low suitability (<0.5) (Fig. 3B) and that such freguesias also lacked historical evidence of WNV presence (Fig. 3C). In contrast, freguesias with intermediate-to-highest suitability (>0.5) included those with and without historical evidence (Fig. 3C). Identifying freguesias with intermediate-to-highest suitability (>0.5) that did not have historical evidence for WNV circulation highlighted the potential of the modelling approach to identify freguesias that, although lacking evidence, hold ecological backgrounds compatible with historical WNV circulation.

Proposing a novel suitability and evidence based framework, we defined three main categories (A to C) of public health importance based on different levels of estimated suitability. We leveraged our estimates of suitability and past evidence of circulation to define suitability thresholds among the categories. Category A, holding the highest likelihood of WNV circulation, was defined for suitability higher than 0.75, which represented the upper 90 % of all estimates (Fig. 3B). Category C, representing the lowest likelihood of WNV circulation, was defined for suitability lower than 0.5, which represented the range for which suitability was lowest and no evidence of WNV circulation existed (Fig. 3C). The remaining range of estimated suitability between 0.5 and 0.75 was used to define Category B for intermediate likelihood of WNV circulation. Categories A and B were further divided into two sub-categories, depending on the existence of past evidence for WNV circulation (Fig. 4). Freguesias with highest suitability distributed to the south (category A, Fig. 4A) represented a broad spatial boundary in which surveillance efforts, such as serological surveys of human and equine, molecular testing of mosquito and viral sequencing, are likely to provide valuable data. Within this boundary, those areas with past evidence of WNV circulation (sub-category A1, Fig. 4A) represented subregions with greatest evidence-based support for active surveillance. In contrast, areas with the main category C mainly distributed to the north (Fig. 4A), had the lowest estimated suitability and lacked historical evidence of WNV (Fig. 3C). These areas highlighted the spatial boundaries where active surveillance is least likely to provide new data, serving primarily to confirm the absence of WNV circulation.

District-wise, Setúbal, Faro and Beja, all in the south, were identified as holding a dominance of freguesias classified with categories A and A1 within their territory (Fig. 4B), thus presenting not just the highest estimated ecological suitability but also the greatest evidence for WNV previous circulation. The district of Évora, although presenting less evidence for circulation, was also highlighted as holding a dominance of freguesias with adequate ecological suitability across its territory. The main and sub-category of each of the 2882 freguesias are provided in Supplementary Table S6.

Towards a One Health perspective, we explored the spatial links between the suitability and evidence based main categories and the three-host axes of WNV surveillance and public and animal health importance – humans, equines and birds. In the main text we focused on identifying the freguesias that presented both the highest suitability

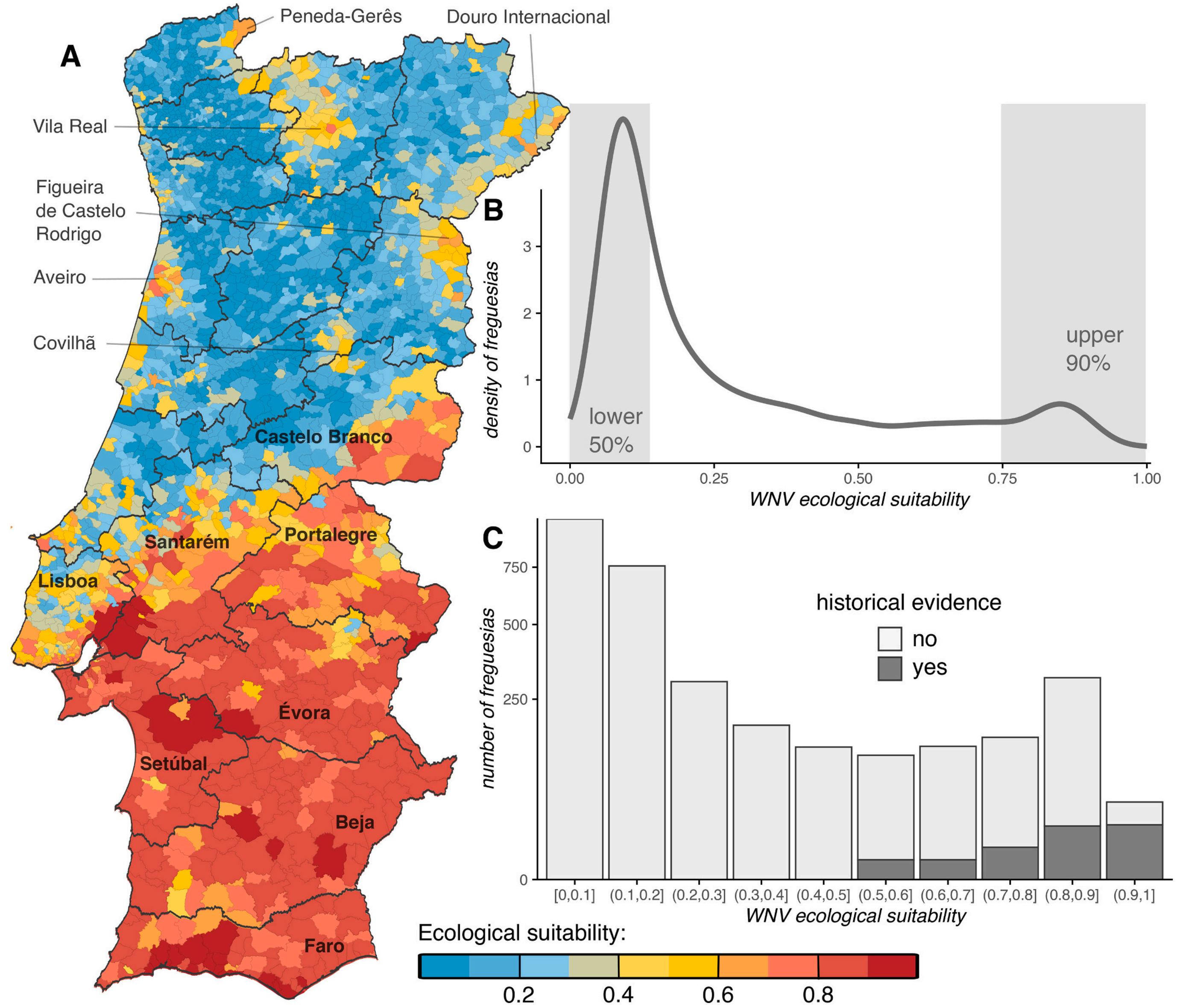


Fig. 3. WNV estimated ecological suitability across Portugal. (A) Spatial distribution of estimated WNV ecological suitability per freguesia (color scale at the bottom). Dark grey spatial borders represent districts. (B) Density distribution of WNV ecological suitability across the country. The shaded area lower 50 % represents the proportion of the distribution under the 50 % percentile (lowest suitability), while upper 90 % represents the proportion of the distribution above the 90 % percentile (highest suitability). (C) Total number of freguesias with suitability within the bins of the x-axis. The number of freguesias with and without WNV historical evidence is presented by the different shades of grey according to the legend.

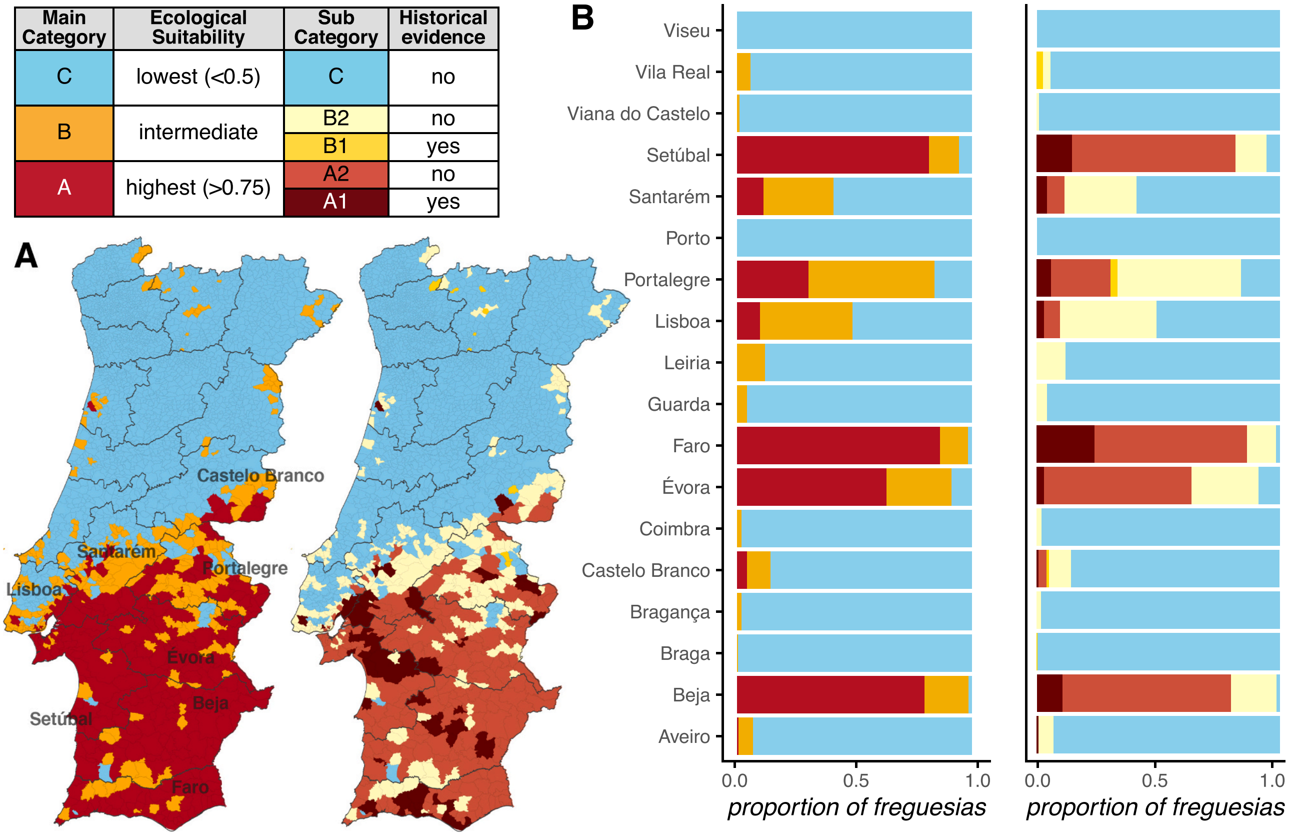
(category A) and highest values of host distributions. A comprehensive bivariate visualization of all main categories and host ranges is presented in Supplementary Fig. 6.

The Portuguese territory is highly heterogeneous in human population density, with the areas of greatest density distributed along the western and southern coasts, with discrete hotspots in the interior (Supplementary Figs. S5–6). Only freguesias in the south were identified as having both the highest suitability and human density (Fig. 5A). The vast majority of identified freguesias belonged to the districts of Setúbal and Lisboa in the south-centre-west, and Faro in the south (Fig. 5B). Notably, two of these districts (Setúbal, Faro) are the ones in which all historical human WNV infections have been reported in the past.

In contrast with the distribution of the human population, with considerably larger densely populated areas in the litoral, the distributions of equines and avian biodiversity are less heterogeneous across the country (Supplementary Figs. S5–6). Overlap between category A and these non-human distributions revealed a number of relevant districts. The top 3 regarding the equine population were Beja, Évora and Santarém, located in the centre-south of the country. For avian biodiversity, the three most relevant also included Beja and Évora, as well as Faro, located in the centre-south and south, respectively. A full list of the identified districts and underlying freguesias in regards to their three-host background and the suitability and evidence based framework is included in Supplementary Table S6.

3.4. *Projecting the impact of climate change*

Climate change has become increasingly evident in Portugal, with the country becoming warmer and drier over the past ~40 years (1981–2019), slowly favouring the theoretical transmission potential of WNV (Lourenço et al., 2022). For example, by analysing satellite climate data for that period, we have previously estimated a 0.04° Celsius yearly increase in mean temperature. We hypothesized that local climate change alone could alter the landscape of WNV ecological suitability. As



Main Category	Ecological Suitability	Sub Category	Historical evidence
C	lowest (<0.5)	C	no
B	intermediate	B2	no
		B1	yes
A	highest (>0.75)	A2	no
		A1	yes

Fig. 4. Proposed WNV ecological suitability and evidence based framework. (A) Spatial distribution of the three main proposed categories (left) and spatial distribution of the five proposed sub-categories (right). (B) Proportion of freguesias within each district that belong to the main (left) and sub-categories (right). (A-B) Both the maps and barplots are colored according to the definitions of the categories as shown on the table (top left).

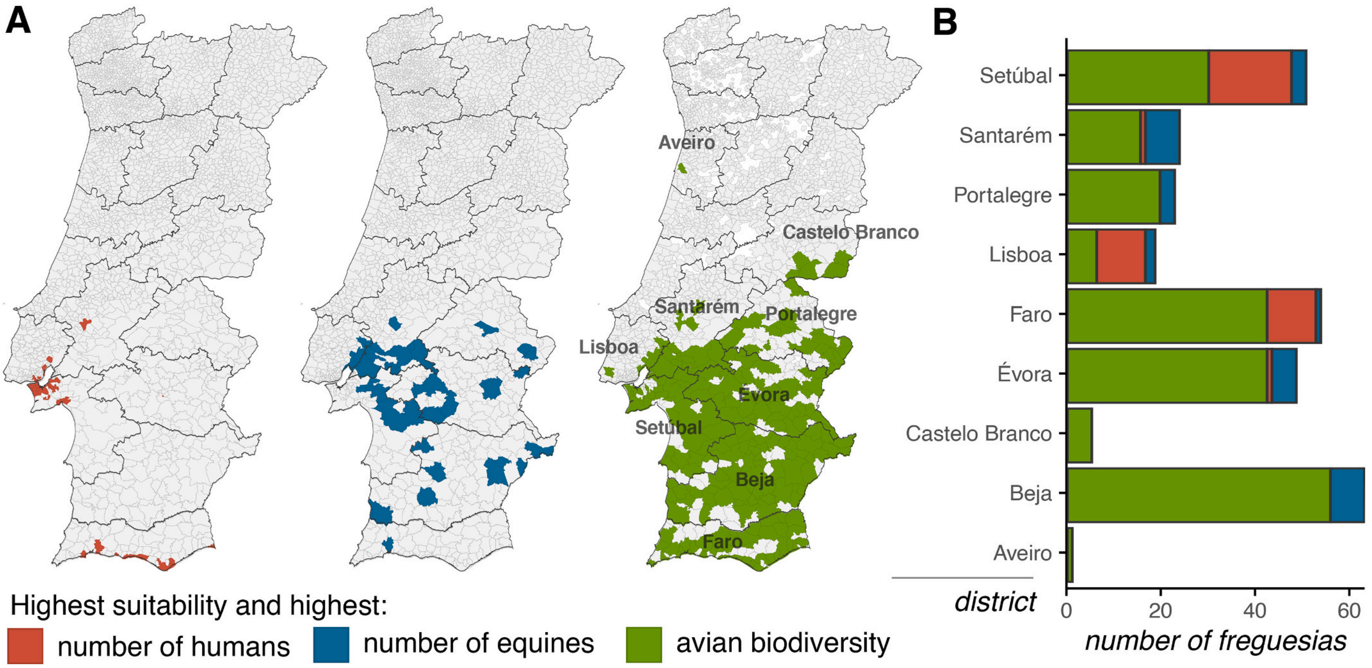


Fig. 5. Overlap between WNV highest suitability and three-host axes. (A) Maps present the *freguesias* that hold both the highest ecological suitability (category A, Fig. 4) and highest number of equines (left), number of humans (centre) and avian biodiversity (right, where white *freguesias* have missing data). (B) Summary of the total number of *freguesias*, per district, presented in panel A. Panels A and B use the color scale presented in the bottom.

a proof-of-concept exercise, we applied the previously published estimates of climate change (Supplementary Text 1) (Lourenço et al., 2022)) to the meteorological variables included in the ensemble model of this study (temperature, precipitation, index P), and projected future changes to suitability up to the year 2050 (Fig. 6).

We found that if climate trends remain similar to those of the past ~40 years, WNV ecological suitability will remain stable across most of the territory, although increasing at specific locations (Fig. 6AB). Overall, we estimated that approximately 62 % of freguesias ($N = 1776$) will experience almost no change (<10 %) in the level of suitability they presented in 2022 up to the year of 2050. Most change would be expected in the north of the country, in one region across the west coast (Lisboa, Leiria, Coimbra, Aveiro, Porto and Braga) and another region across the north-south spatial boundary of the currently suitable south (Santarém, Castelo Branco) (Fig. 6B). Approximately 6 % of freguesias ($N = 179$) will experience decreases in suitability, while 32 % ($N = 927$) will experience an increase. Decreases, however, were estimated to be only marginal in intensity when compared to increases (Fig. 6A). Among freguesias with estimated increases by the year 2050, suitability will increase on average by 66 % above the current values, but critically, 194 of these will experience more than a doubling (+100 %) of their current suitability (Fig. 6A).

Following these trends, climate change will result in some freguesias changing from category C (lowest suitability) to category B (intermediate suitability), and others from category B to category A (highest suitability). Both the total number of freguesias with categories B and A will grow over the next decade (Fig. 6CD). Those with category B will grow in number from 245 in 2022 to 341 (range 305–364) in the year 2050 (Fig. 6C), while those with category A will grow from 263 in 2022 to 321 (range 293–372) (Fig. 6D). Critically, while the average number of freguesias changing to category B is predicted to be higher, the change in land area by category A will be larger. Effectively, by 2050 category B freguesias will represent +0.8 % of land area compared to 2022, while category A freguesias will represent +5.5 % of land area, equating to +489 K hectares (Supplementary Fig. S7). The vast majority of changes in main categories (C to B, and B to A) will take place in freguesias standing on the spatial boundaries of the current north-south divide in suitability (Supplementary Fig. S8).

4. Discussion

Portugal, standing on the far south-west of Europe, remains an outlier among its Mediterranean peers on not having yet experienced a WNV epidemic associated with human populations. To date, only four human cases have been reported, even with accumulating evidence that WNV circulates in the country and tangentially spreads from its avian-centred transmission cycle into equine populations. Expanding previous attempts to decipher the uniqueness of the Portuguese context, we here apply ecological niche modelling based on machine learning techniques informed by an historical WNV evidence dataset

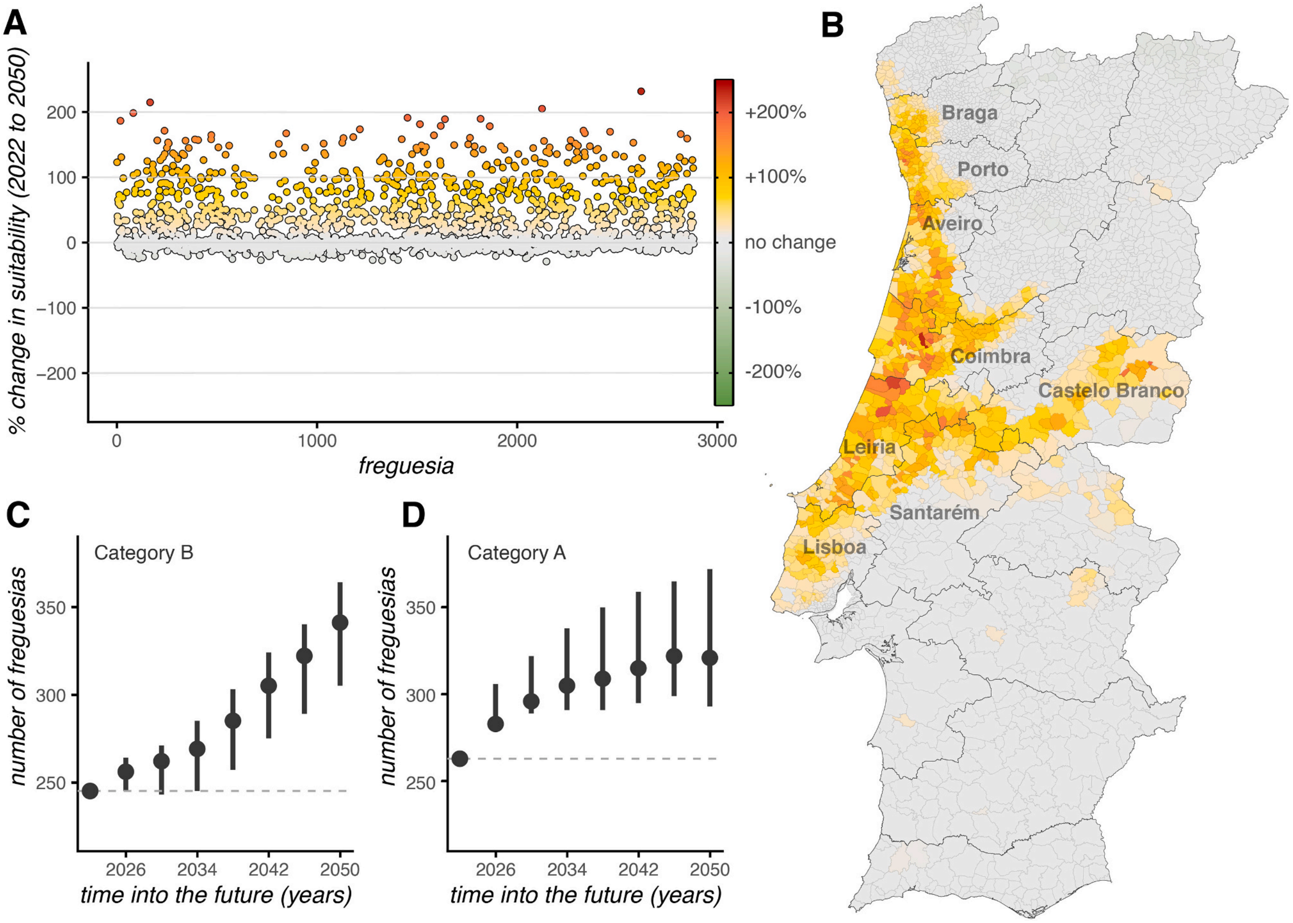


Fig. 6. Contribution of climate change to suitability and evidence based categories. (A) Percent change in suitability per freguesia between the years 2022 and 2050. The x-axis presents all 2882 freguesias without any specific order. Freguesias (points) are colored according to whether suitability increased (warmer colors), remained mostly unchanged (grey), or decreased (colder colors). (B) Spatial distribution of freguesias presented in panel A. (A-B) Panels A and B share the color scale presented in panel A. (C) Change in time of the total number of freguesias holding category B as defined in Fig. 4. (D) Change in time of the total number of freguesias holding category A as defined in Fig. 4. In panels C—D, the whiskers represent the range of projections given the uncertainty in the rates of change of each climatic variable (see main text for source of rates).

(1969–2022) and a large number of environmental variables ($N = 51$) from across $N = 2882$ freguesias.

Leveraging the modelling outputs, we show that ecological backgrounds compatible with past evidence for WNV circulation are mostly restricted to the south of the country, generally defined by a warmer and drier climate, the presence of higher avian diversity and a combination of unique avian species and land types. Although the modelling approach can not provide evidence of causation, these results are in line with existing knowledge regarding the zoonotic cycle and spillover dynamics of WNV. For example, temperature is the most explored and validated climatic variable driving the seasonality and spatial distribution of WNV incidence (García-Carrasco et al., 2023; Paz, 2015; Reisen et al., 2006). The observation that the estimated likelihood of WNV circulation is lowest below 15C and increasingly higher as temperature rises is interesting, since temperatures around this value commonly feature as a critical threshold for various mosquito traits (e.g. lifespan, biting rate, egg-to-adult survival) and WNV replication in mosquitoes or culture in empirical data resulting from laboratory controlled experiments on species of mosquito, including *Culex* (Di Pol et al., 2022) and *Aedes* spp. (Mordecai et al., 2017). Our findings of suitable ecological backgrounds defined by low precipitation is in line with existing studies (Farooq et al., 2022; Myer and Johnston, 2019; Magallanes et al., 2023), but contrasts with others (Soverow et al., 2009). In general, reported relationships between WNV activity and precipitation include contrasting conclusions, which is a likely result of a complex landscape of factors, including that precipitation is patchy in spatio-temporal behavior (compared e.g. with temperature), and that mosquito populations from different regions vary in their reaction to precipitation-induced biological queues (Paz and Semenza, 2013). In the context of reservoir hosts, among the small number of species explored in detail, we identify a group of interest for their associations with historical WNV circulation: *Common Blackbird, Eurasian Jay, Barn Swallow, European Robin, Mallard, Eurasian Magpie, Common Wood Pigeon, White Stork*, and the *Common Cuckoo*. Two groups of avian species, aggregated as migratory (*White Stork, Barn Swallow*) and belonging to the Corvid family (*Carrion Crow, Northern Raven, Eurasian Magpie, Eurasian Jay*) have also been identified as being of interest. Findings related to the link between avian biodiversity and WNV activity are contradictory in the literature. Some studies have suggested a negative relationship between the two (Ezenwa et al., 2006; Allan et al., 2009), whereas others, similarly to the current study, suggest a positive relationship (Levine et al., 2017; Casades-Martí et al., 2023; Ferraguti et al., 2021a, 2021b). One reason why diversity measures often result in contrasting evidence may be that such measures do not take into consideration community composition (Ferraguti et al., 2021a), such that e.g. two regions with similar diversity may contain completely different species that potentially differ in WNV susceptibility and transmissibility phenotypes (Gamino and Höfle, 2013; Wheeler et al., 2009). Indeed most avian species are not highly competent for and contribute minimally to WNV transmission, such that community composition remains critical on the background of community diversity. In our study, the distributions of specific species scored lower in importance than biodiversity, suggesting that community composition was less related to historical WNV circulation than biodiversity. Similarly, the notable prominence of the generalized land type predictor, in contrast to any specific land type predictor, could be attributed to the known contribution of multiple land types to WNV transmission success (Paz and Semenza, 2013). We were nonetheless able to find positive associations of WNV ecological suitability to specific land types, mainly those related to the presence of water bodies (e.g. wetlands, dunes, salt marshes, estuaries) or irrigated land (e.g. golf courses, agroforest, green urban). Although such associations vary by region of study, many have been commonly reported both in Europe (Hubálek and Halouzka, 1999; Pradier et al., 2014) and elsewhere (Gates and Boston, 2009).

Spatially, we also derived useful insights. The identified geographical, north-south boundary separating the lowest and highest suitability regions closely aligned with the demarcation of two existing climates types. To the west, within the district of Lisboa with the capital city and the Tagus River, is a national landmark for bird migration hotspots and avian biodiversity. Indeed, areas surrounding the capital were identified with high ecological suitability, going against the established assumption that, nationally, WNV favorable ecological conditions should be limited to the inner, rural and low human density areas of the country. We also identified regions with suitability that typically do not feature among those with past evidence for WNV circulation. For example, the area around the city of Aveiro contains the ecologically significant wetland Ria de Aveiro with a local high richness of nesting bird species. The localities of Figueira de Castelo Rodrigo, Peneda-Gerês and Douro Internacional, near protected natural parks, also contained suitable areas for WNV transmission. Finally, some of the identified localities with highest ecological suitability belonged to the districts of Setúbal (south-west) and Faro (furthest south). Among these were the localities where the only four human cases have been reported in Portugal. For example, Ria Formosa in Faro, the approximate location of two cases that occurred in birdwatchers, is an avian sanctuary that holds large numbers of resident and migratory species, making it a popular site for bird watching. Since the south of the country drives the national tourism industry, both the concentration of previous evidence and higher ecological suitability for WNV circulation in the south further highlight a public health risk to international travelers. The only large-scale arboviral epidemic in Portuguese territory (dengue on the Island of Madeira (Lourenço and Recker, 2014)) resulted in significant local tourism-related economic loss, and lessons from this experience should be transferred to continental territory. Awareness campaigns targeting not just the local population but tourists as well, for example focusing on prevention measures and dispensing repellents to reduce exposure to mosquito bites, could be of value in the near future as WNV activity intensifies in the Iberian peninsula.

We considered 51 environmental variables but performed recursive feature selection to reduce the number of variables in light of the limited, existing WNV circulation data. We decided on a conservative approach to limit modelling error, retaining 19 variables for analyses. Although the 19 variables described the historical ecological backgrounds of WNV and included factors not studied in the Portuguese context to date, the exclusion of variables was also informative on its own. For example, variables specific to human and other mammals (e.g. ovines, bovines) were excluded, validating the notion that transmission, wherever it has occurred, is not dependent on mammals, even if these animals can experience viral spillover. Of the climatic variables, only humidity was excluded, pointing to the fact temperature and precipitation typically hold sufficient information to capture the role of local climate. In general, modelling studies tend to focus on the roles of temperature and precipitation, and the specific role of humidity remains less studied (Paz, 2015). Nonetheless, this outcome is in line with a study in Israel, in which seasonal humidity was shown not to be correlated with multi-year WNV human incidence, while both temperature and the index P (including humidity as input) were highly correlated (Lourenço et al., 2020). Similarly, identifying temperature and precipitation as more informative is in line with other modelling revealing significant relationships of WNV occurrence with temperature and precipitation (although with opposite correlation trends) (Farooq et al., 2022; Myer and Johnston, 2019; García-Carrasco et al., 2023). Variables specific to certain avian species were also excluded, including *Corvus corax* (Common Raven), *Turdus philomelos* (Song Thrush), *Parus major* (Great Tit), *Corvus corone* (Carrion Crow), *Passer domesticus* (House Sparrow), suggesting low potential for future surveillance in these species in Portugal.

Given ongoing reports across the globe on the effects of climate change on the epidemiology of mosquito-borne viruses due to the impact of temperature increases in the vector side of transmission cycles (e.g. increasing viral replication rates or frequency of blood feeding), we hypothesized that climate change alone could alter the current WNV ecological suitability in Portugal. For this we used the ensemble model to project suitability up to the year 2050 when applying previously estimated rates of change to the three climate-related variables informing the model. In general, we estimated that climate change alone could push the current north-south suitability geographical divide northwards, while keeping the south as the most suitable region. Climate change trends pushing the boundaries of mosquito-borne suitability northwards, specially in Europe and North America, is a topic of much ongoing research with a vast amount of supportive epidemiological and entomological evidence (Ainsworth, 2023). Critically, the largest trends towards higher WNV suitability in Portugal up to the year 2050 would take place along the west coast where a large proportion of high density human populations are localized. Climate change and demographics may thus coincidently raise the proportion of the human population at risk of WNV spillover into the near future.

An open question in Portugal pertains to whether the virus is endemic or epidemic (i.e. introduced annually or persisting over seasons). While we do not provide a definite answer, we generate sufficient output to propose that endemicity, if possible, would likely be favoured to the south. This is in line with mounting evidence from Spanish research and surveillance initiatives, including genomic data, strongly in favour of WNV endemicity in Southern Spain, bordering Southern Portugal (García-Bocanegra et al., 2022; Casimiro-Soriguer et al., 2021; Macias et al., 2023). Our dataset also includes a noteworthy evidence point from the south, involving a symptomatic horse that tested positive for WNV antibodies off-season (on January 26, 2022) after reports of other symptomatic equines in the freguesia of Comporta, Setúbal district. Our estimation of the ecological suitability for that freguesia is one of the highest in the country at 0.89. Together, this epidemiological event and our modelling output suggest potential, local overwintering of the virus. Support for endemicity could be tested in the future by the identification of unique, local viral variants, but to date this has not been feasible, with only two genome sequences available (Mencattelli et al., 2023). From our proposed suitability and evidence based framework, we pinpoint districts (and their specific freguesias) where animal seroprevalence surveillance efforts are urgently needed, and where focusing on animal carcases or living animals with compatible symptoms to WNV infection would more likely result in positive molecular evidence and thus new isolates for sequencing. Focus could be, for example, on freguesias identified as sub-category A1 (high suitability with past evidence) and high equine density or high avian biodiversity, during typical months of WNV occurrence (May to October).

In parallel to animal surveillance, mosquito surveillance in Spain (García San Miguel Rodríguez-Alarcón et al., 2021; Eritja et al., 2021; Figuerola et al., 2022) and Italy (Mingione et al., 2023; Rizzo et al., 2016; Calzolari et al., 2022) has demonstrated benefits in the early detection, characterization and understanding of local WNV epidemic activity. In Portugal, active mosquito surveillance implemented since 2008 has not produced similar outcomes. It has, nonetheless, been successful at monitoring and characterizing the spatial distribution of invasive species (e.g. *Aedes aegypti*, *Aedes albopictus*) and of resident, WNV-transmitting *Culex* spp. species. Evidence has accumulated over the years on the spatial distribution of *Culex* spp. across the territory, including *Culex pipiens*, *Culex univitattus / perexiguus*, *Culex theileri* and *Culex modestus*, with the latter seemingly being the only species not widespread from north to south (see e.g. the surveillance report for 2022 (REVIVE, n.d.). The suitability and evidence based framework proposed in this study focuses on the animal dimension of surveillance. Nonetheless, this should not be seen as not supporting mosquito surveillance nationally. In fact, the widespread presence of *Culex* spp. implies that districts and freguesias of highest value for mosquito surveillance can be guided by the framework presented in Fig. 4. In particular, mosquito surveillance should focus on freguesias identified as category A, in combination or not with any of the output related to the three-host axes.

There are limitations to this work, mostly related to currently available data, that should be revisited in future research once better and complementary data exists. One is the predominance of equine-related evidence in the dataset. Although a consequence of the epidemiological history and passive surveillance in Portugal, it is possible that our outputs are biased to identifying ecological backgrounds that more specifically support spill-over events to equine populations. Nonetheless, it is reassuring that the equine variable was given far less modelling importance than climatic, land type and avian biodiversity variables, and that estimated ecological suitability was mostly restricted to the south in contrast to the equine distribution that included the entire north-south range. At the same time, these results also follow the expectation that other factors beyond equine presence or abundance should drive the transmission potential of WNV given that equines are dead end hosts. There is also the possibility that the 59 freguesias with WNV presence status do not represent the real spatial range of WNV circulation in Portugal due to the current deficiencies in surveillance. If this ought to be the case, then the models would be overfitting the data by learning from a limited set of suitable ecological backgrounds common to these 59 freguesias, when in reality the characteristics of suitable backgrounds would be broader. It remains nonetheless reassuring that the spatial distribution of our suitability estimates is remarkably similar to those of various other studies that have used much larger WNV circulation data (e.g. (Durand et al., 2017; García-Carrasco et al., 2023; Di Pol et al., 2022; García-Carrasco et al., 2021)). In effect, although suitability landscapes can be similar across different modelling studies, studies contribute to insight generated about variables not explored in other studies (in our case, e.g. avian-related variables, land types, index P). Other limitations include the use of variables measured from different time periods, a necessity given the scarcity of better sources for variables of interest. For example, the data on avian species provided by eBird was restricted between March and May (2015–2021). Reasons for this include the fact that birds are less mobile and observation effort is high during this period, providing a more robust evaluation of the spatial distribution of species (including resident, and those migrating into the country in spring). At the same time, while eBird remains the most complete and rich data set on avian species in Portugal, the data includes challenges related to its representativeness. eBird data is composed of thousands of observation records that vary significantly in terms of location and allocated observation time. This inherent spatio-temporal variation in coverage makes it difficult to assess, for example, if existing records of particular species in some locations is truly representative of the actual abundance or a reflection of observational effort. In an ideal scenario, using solely WNV circulation evidence related to resident avian species would more directly associate suitability outputs with local transmission events. However, given the already limited amount of data, we included evidence related to both resident and migratory species, such that the suitability outputs should be interpreted as a mixture of possible local (among resident species) and onwards (from migratory to local species) transmission potential. We further note that most of the WNV occurrence data related to avian hosts was based on serology, implying survival past WNV infection. However, it is known that susceptibility to disease and death risk due to WNV infection can vary substantially between avian species, such that WNV-infected dead birds can serve as a sentinel source for surveillance (Nielsen and Reisen, 2007). While our historical WNV occurrence dataset did not include evidence related to this, we note that the modelling outputs identify the localities where surveillance focused on dead birds could be implemented. In terms of methods, we selected BRT and RF for being common in ecological studies and for having expertise with them. The RF showed a marginally better capacity in discriminating historical WNV presence-absence, which is consistent with other studies (e.g. (Richard Cutler et al., 2007)). While the minor discrepancies in output between the models are expected, exploring alternative models, specially in light of new data, could be fruitful in the future. Regarding the projection of future climate effects, we did not include interactions with other factors. It is plausible that future climate trends will also affect other factors that were identified as drivers of suitability (e.g. avian biodiversity or the predominant local land type). As such, our results serve merely as a demonstration that climate change alone can alter and indeed expand the spatial boundaries suitable for WNV transmission, but the interaction with, and contribution of other factors should be addressed in future analyses. Finally, the outputs described in the study relating input variables with estimated suitability are demonstrative of association and not causation.

Our study contributes with a novel and unique perspective on the past, present and future ecology of WNV in Portugal, providing first of a kind valuable insights for decision-making into the future. From a One Health perspective, several districts are identified as holding adequate combinations of ecological suitability, past evidence and the presence of relevant hosts. Lisboa, in the west-centre region, for example, represents an almost virgin ground regarding WNV surveillance, but emerges as critical for future zoonotic spill-over potential into human populations. On the other hand, Faro in the far south, holds an optimal combination of factors that present opportunities to survey WNV current incidence in humans and equines, and circulation in birds. Other districts such as Beja and Évora, instead, present opportunities for future studies focused mostly on non-human animals. The identified districts and underlying freguesias with varying relevance regarding the three-host axes form the initial basis of an information-driven framework under which future One Health surveillance should be based in Portugal.

Through efficient surveillance, geographical areas of risk can be identified and viral presence can be early detected, allowing for the implementation of timely interventions and mitigation measures both in humans and other animals. Active surveillance should include vector, human and other animal monitoring, as well as health education initiatives to raise veterinary, clinical and public awareness to WNV. Italy provides a good example of an integrative One Health surveillance scheme with recent success (Mingione et al., 2023). A shift towards a similar active surveillance with inclusion of genomic surveillance is recommended for Portugal in the near future. This shift can be guided by the suitability and evidence based framework proposed in this study. Only this way can we close existing gaps in knowledge, enhance our understanding of the evolving emergence of WNV, and be prepared to respond to the first and subsequent human-associated epidemics in the country.

In summary, using modelling techniques informed by the most up-to-date data available for Portugal, we unveil that ecological backgrounds compatible with WNV circulation are likely associated with a warmer and drier climate, higher avian biodiversity, the presence of specific avian species (e.g White Stork, Common Cuckoo or Barn Swallow) and particular landscapes (e.g. wetlands, estuaries or irrigated land). Such suitable backgrounds are found to be concentrated to the south of the country, neighboring cross-border regions of Spain where in recent years WNV has been reported to have significantly increased in epidemic activity. Built on evidence and modelling outputs, we develop the first category-based spatial framework that identifies localities, stratified by host-types (human, avian, equine), that are of most relevance for surveillance, awareness and control initiatives towards mitigation of human (local, touristic) and animal health impact of the expected future increase in WNV activity in the country. We further show that climate change may be expected to shift the geographical boundary of suitable areas northwards and along the west coast, in a similar fashion to what is predicted across Europe. Our results provide a holistic perspective on the past, present and future ecology of WNV in Portugal, under a quantitative framework that unravels opportunities to prepare and respond to the first human-associated epidemic in the country.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.173875.

CRediT authorship contribution statement

Martim A. Geraldes: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Mónica V. Cunha:** Writing – review & editing, Writing – original draft, Supervision, Resources, Formal analysis, Data curation, Conceptualization. **Carlos Godinho:** Writing – review & editing, Data curation. **Ricardo F. de Lima:** Writing – review & editing, Data curation. **Marta Giovanetti:** Writing – review & editing, Supervision. **José Lourenço:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

All data is provided as Supplementary Data File 1 (Excel), including WNV historical evidence (Table S1), details on the 51 predictive variables considered (Table S2), values of the 51 predictive variables considered per freguesia (Table S3), estimated ecological suitability per freguesia (Table S4), categories per freguesia for the suitability and evidence based framework (Table S5), relevance status per freguesia related to the 3-host framework (Table S6).

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