



A systematic review of environmental factors related to WNV circulation in European and Mediterranean countries

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ABSTRACT

Introduction/objective: West Nile virus (WNV) is one of the most widely distributed flaviviruses worldwide. It is considered an endemic and emerging pathogen in different areas of the Europe and Mediterranean countries (MR). Mosquitoes of the genus *Culex* spp. are the main vectors, and birds its main vertebrate hosts. It can occasionally infect mammals, including humans. Different environmental factors can influence its distribution and transmission through its effects on vector or host populations. Our objective was to determine environmental factors associated with changes in vector distribution and WNV transmission in Europe and MR.

Material & methods: Systematic peer review of articles published between 2000 and 2020. We selected studies on WNV, and its vectors carried out in Europe and MR. The search included terms referring to climatic and environmental factors.

Results: We included 65 studies, of which 21 (32%) were conducted in Italy. *Culex* spp. was studied in 26 papers (40%), humans in 19 papers (29%) and host animals (mainly horses) in 16 papers (25%), whereas bird reservoirs were addressed in 5 studies (8%). A significant positive relationship was observed between changes in temperature and precipitation patterns and the epidemiology of WNV, although contrasting results were found among studies. Other factors positively related to WNV dynamics were the normalized difference vegetation index (NDVI) and expansion of anthropized habitats.

Conclusion: The epidemiology of WNV seems to be related to climatic factors that are changing globally due to ongoing climate change. Unfortunately, the complete zoonotic cycle was not analyzed in most papers, making it difficult to determine the independent impact of environment on the different components of the transmission cycle. Given the current expansion and endemicity of WNV in the area, it is important to adopt holistic approaches to understand WNV epidemiology and to improve WNV surveillance and control.

1. Introduction

West Nile Virus (WNV), present in large parts of Europe, America, Africa, Asia, such as the Middle East, is one of the most widely distributed vector-borne flaviviruses in the world. *Culex* spp. mosquitoes are the main vectors of WNV [1]. Birds are the main vertebrate reservoirs of the virus. Most mammal species, including humans, are not able to reach sufficiently high viremia levels to infect blood-feeding mosquitoes and

consequently, are considered as dead-end hosts for WNV infection [2]. Bird migration is considered as an important factor for WNV dispersal and emergence [3].

Nowadays, WNV disease (WND) is considered endemic in many countries of Europe and emerging in others [4]. In 2020 and 2021, European Union (EU) and European Economic Area (EEA) countries reported 185 and 43 outbreaks among equids and 2 and 8 outbreaks in birds, respectively. The outbreaks were declared in Spain, Germany,

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Table 1
Eligibility criteria.

Inclusion criteria	Exclusion criteria
Original research studies Studies must refer to environmental factors	Other type of study Not assessing the impact of environmental factors on WNV
Published between 01/01/2000 and 31/12/2020	Published before 01/01/2000 or after 31/12/2020
Languages: English, French, Portuguese, Italian, German, and Spanish	Not carried out in Europe and/or Mediterranean countries.

Italy, France, Portugal, Austria, Bulgaria, Greece, Hungary and Slovenia [4].

Human cases of WND have increased in the last decades. In 2018, there was a 7.2-fold increase in the number of human cases compared to the previous seasons [5]. In 2020, EU/EEA countries reported 316 human cases, most of them in Mediterranean countries [4], while autochthonous human cases were reported for the first time in Germany and the Netherlands [6,7].

Environmental factors may influence WNV transmission dynamics through their effects on both vector and/or host populations [8]. Environmental factors modulate vector presence and abundance, the extrinsic incubation period (EIP) [9], and human-derived changes in landscape may provide favorable conditions for mosquito breeding and maintenance due to the capacity of some species to exploit small water sources, mostly in urban areas [10].

An increase in WNV transmission has been observed in the Mediterranean Region (MR) over the last years [11], but data are scattered and the ultimate factors associated with this apparently generalized trend remain unclear. An integrative view of all mentioned factors (vectors, reservoirs, and the potential interactions between them) is still necessary to fully understand WNV dynamics and epidemiology. This systematic review aimed at identifying which and how environmental factors influence the presence and/or distribution of vectors and WNV circulation in mosquitoes, avian reservoirs, non-human and human hosts in EU and neighboring MR countries.

2. Material and methods

A systematic review was performed between June 1st, 2021, and June 30th, 2021 on the influence of environmental factors and their changes on WNV presence, dynamics and epidemiology in EU and MR in the last two decades following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for systematic reviews and meta-analyses [12]. The study protocol was registered in the PROSPERO (<https://www.crd.york.ac.uk/PROSPERO/>) and was accepted on May 16th, 2021 (PROSPERO ID CRD42021235292). To evaluate internal and external validity of the selected publications, a 12-items quality assessment tool was used and adapted (supplementary table 1). Quality was scored as a binary variable (yes/no) and the sum of 1 (“yes”?) gave the final evaluation. The complete search strategy and quality assessment are detailed in supplemental material 1.

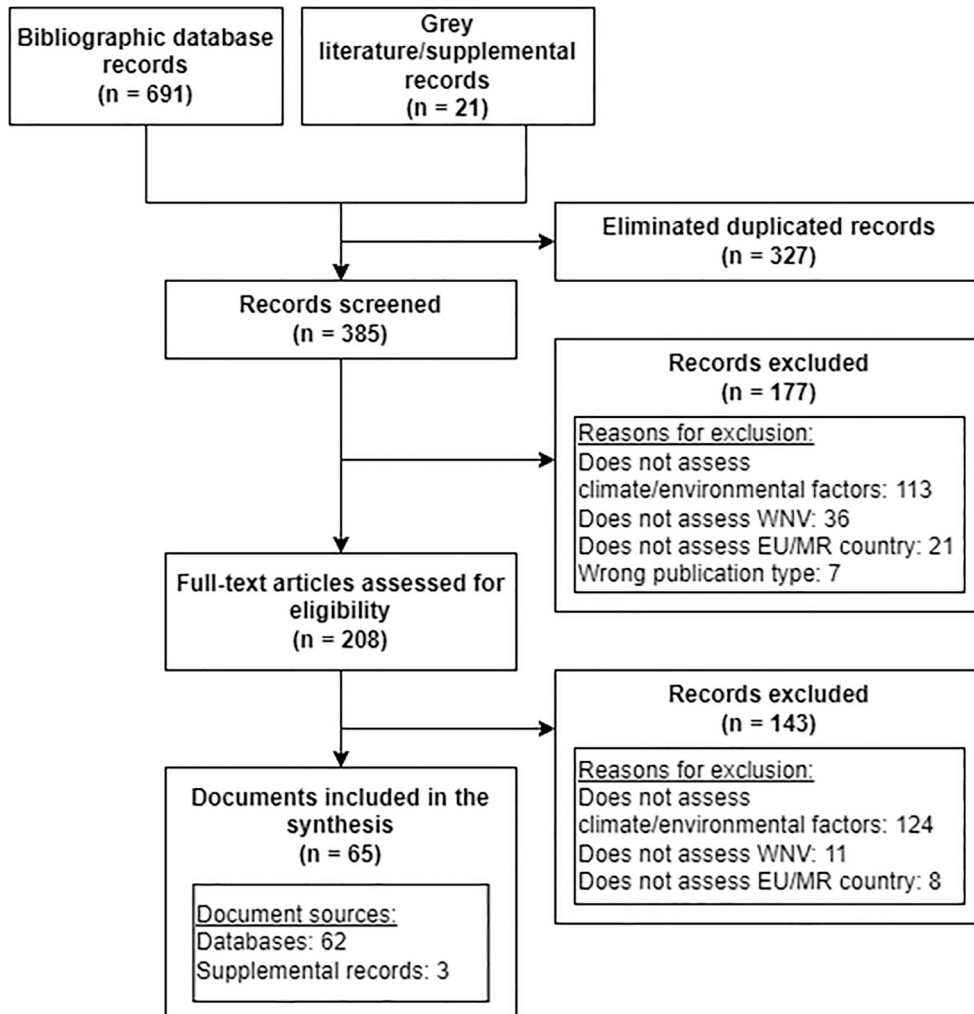


Fig. 1. Study selection process.

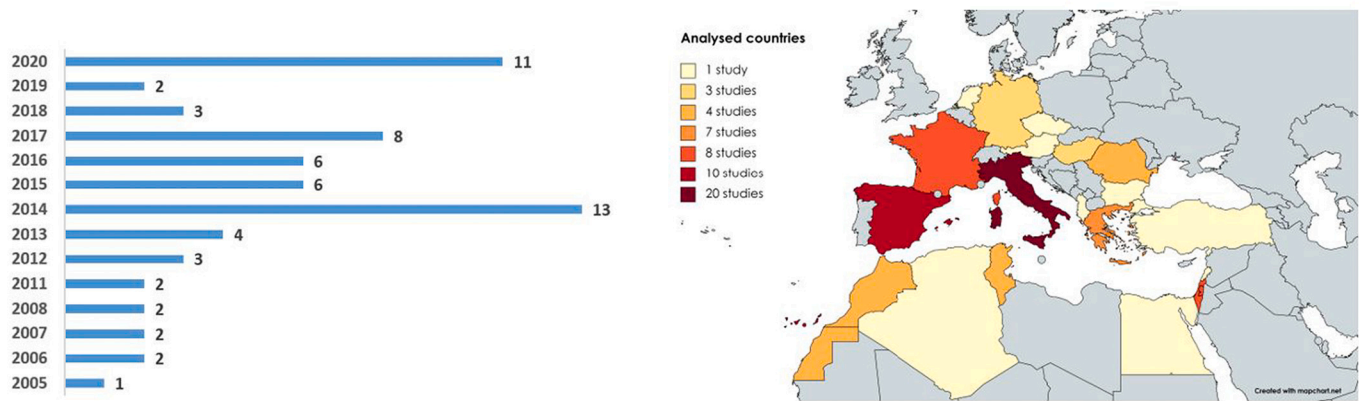


Fig. 2. Articles by year of publication (left) and analyzed countries (right).

2.1. Eligibility criteria

The search was performed in English, French, Portuguese, German, Italian and Spanish. Only original research studies with quantitative analysis were considered. Both proxy (i.e., vector presence and abundance) and host/reservoir population (birds, humans, equids) were considered as WNV activity indicators. Inclusion and exclusion criteria are shown in Table 1.

After the exclusion of duplicate citations, two independent reviewers screened titles and abstracts, followed by full-text reviews.

A PRISMA flow-chart summarizing the search and inclusion/exclusion process was produced (Fig. 1).

To evaluate internal and external validity of the selected publications, a 12-items quality assessment tool was used and adapted (supplementary table 1). Quality was scored as a binary variable (yes/no) and the sum of 1 (“yes”?) gave the final evaluation.

2.2. Data collection and analysis

The selected papers were systematically analyzed. Each reviewer read all the selected articles and entered the information into a concept database, including identifier, reference, first author, year of publication, vector, disease, reservoir, host, country of the study, year of study, study design, environmental and/or climatic factor and data sources, analytical approach (association/correlation models, density/abundance models, predictive models, spatial models, transmission models), summary of the results, impact on WNV incidence (Yes/No) and projected prevalence.

Studies were classified based on vectors, reservoirs or animal and human hosts, or if they addressed WNV circulation in general.

References retrieved were saved in Zotero software 5.0.67 (www.zotero.org). We also used the freely available systematic review tool Rayyan (<https://www.rayyan.ai/>) for listing the selected papers and Canva (www.canva.com) and Mapchart (<https://www.mapchart.net>) to produce figures and maps.

3. Results

The systematic search strategy yielded 712 citations. After screening the titles and abstracts, we retained 208 articles for full-text screening. A final set of 65 articles met all inclusion criteria and were included in our review [10,13–76].

3.1. Descriptive characteristics of the studies

Most papers ($n = 58$) were published in 2010–2020 (Fig. 2). Eight studies covered more than one country. The most frequently used types of models were association/correlation models ($n = 41$) and predictive

models ($n = 29$). Other models were spatial models ($n = 7$), density/abundance models ($n = 4$), and transmission models ($n = 1$). Seventeen studies used two different types of modelling approaches (Table 2). Only six studies reported the WNV lineages, of which three studies detected lineage 2 in Greece [20] and Germany [39,74]; and another three identified lineage 1 in Spain [15,42] and Morocco [48]. Several studies conducted predictive models for WNV dynamics (supplementary table 6).

3.2. West Nile virus vectors

Thirty-four studies addressed the impact of environmental factors on WNV vectors (supplementary table 2). Temperature was the most frequently analyzed environmental variable. It was considered a key driver for WNV vector presence [47,55,57,60,63,65], abundance [22,28,29,32,35,46,50,51,59,62,64,66,69,70,72] or both [17,54]. In addition, different studies analyzed the relationship between temperature and mosquito population growth rates [31,36] and mosquito infection rate [38]. Increases in mean, maximum and minimum daily [29,64], weekly [50,64,66], biweekly [36], monthly [15,23,35,36,55,65,69,72] or annual [50,66,69,72] temperature were positively related to the abundance of *Cx. pipiens* [22,28,29,35,36,46,50,54,62,64,66,69,70,72] and other *Culex* species [15,23,32,50,55,59,62,65,66,72], *Ochlerotatus* spp. [66,70] or *Aedes* spp. [70] (Fig. 2). An increase of 1.5–2.5 °C in average temperature from April to June led to higher adult *Cx. pipiens* populations, whereas a decrease showed the opposite effect [55]. Other studies showed that higher temperatures early in the season were followed by earlier and longer vector’s season [29,35,55], especially when higher temperatures occurred between early spring and June [29,35,55]. On the other hand, if the temperature increase occurred later, a shorter vectors’ season and lower vector abundances were observed [35,64]. In the areas with higher temperatures the relationship with abundance was not linear because abundance may be reduced at the higher range of temperatures [62,77].

Precipitation was another driver for WNV vector presence [17,47,60,63,65], abundance [22,28,29,32,35,46,50,51,59,62,64,66,69,70,72] or both [34,54], WNV presence in mosquitoes [23,32,60] and mosquito population growth rates [31,36]. An association was observed between increased cumulative daily precipitation in the 15 days prior to mosquito capture [31,36,50,64–66], increases in daily [31,36], weekly [50] or monthly [64–66,72] precipitation and increased *Cx. pipiens* abundance and growth rates [31,36]. *Culex* spp. abundance was higher if average rainfall between June and July was higher than usual [35,64]. Similarly, if the rainy season was delayed 10 to 30 days, mosquito populations decreased, as the vectors’ season shortened [50,59].

Sixteen studies assessed WNV vectors in relation to presence or distance to certain sites that may function as vector breeding sites

Table 2
Characteristics of the papers included in this study.

ID	First author	Year of publication	Analyzed vector (species)	Analyzed reservoirs & hosts	Genetic lineage	Analyzed countries	WNV indicator	Analytical approach	Study quality
1	Azmi K (27)	2017	ND	Infected horses, donkeys, mules, goats, sheep, camels	ND	Israel	Animal cases	AM	11
2	Coroian M (28)	2020	ND	Horse and bird populations, infected humans, human population	ND	Romania	Human cases	PM	11
3	Sánchez-Gómez A (29)	2017	Infected <i>Cx. pipiens</i>	Infected birds, horses, and humans	ND	Spain	Bird, horse, human cases, virus presence in mosquitoes	AM, PM	11
4	Moirano G (30)	2018	ND	Infected humans	ND	Italy	Human cases	AM	12
5	Gangoso L (31)	2020	<i>Cx. pipiens</i> presence	ND	ND	Spain	Mosquito presence	AM, PM	12
6	Tran A (32)	2014	ND	Infected humans	ND	Europe	Human cases	AM, PM	9
7	Aharonson-Raz K (33)	2014	ND	Infected horses	ND	Israel	Horse cases	AM	12
8	Ladbury GAF (34)	2013	ND	Infected humans	Lineage 2	Greece	Human cases	PM	8
9	Bargaoui R (35)	2015	ND	Infected horses	ND	Tunisia	Horse cases	PM	12
10	Groen TA (36)	2017	<i>Cx. pipiens</i> abundance	ND	ND	France, Greece, Italy	Mosquito abundance	PM	9
11	Calzolari M (37)	2015	Infected <i>Cx. pipiens</i> , <i>Aedes vexans</i> , <i>Oc. caspius</i>	ND	Lineage 1 & 2	Italy	Virus presence in mosquitoes	AM, SM	12
12	Paz S (38)	2006	ND	Infected humans	ND	Israel	Human cases	AM	12
13	Conte A (39)	2015	Mosquito seasonality	Infected humans and horses	ND	Central Europe and Mediterranean Basin	Human, horse cases	AM, PM	12
14	Ben Hassine T (40)	2014	ND	Infected humans	ND	Tunisia	Human cases	AM	10
15	Valiakos G (41)	2014	ND	Infected birds	ND	Greece	Human cases	AM, SM	10
16	Paz S (42)	2008	Infected <i>Cx. pipiens</i> , <i>Cx. pipiens</i> abundance	WNV frequency in humans	ND	Israel	Mosquito abundance	AM	12
17	Marini G (43)	2016	<i>Cx. pipiens</i> abundance	ND	ND	Italy	Mosquito abundance	DM	10
18	Lourenço J (44)	2020	Mosquito parameters	Infected humans	ND	Israel	Human cases	AM	10
19	Fornasiero D (45)	2020	<i>Cx. pipiens</i> abundance	ND	ND	Italy	Mosquito population growth rates	AM	12
20	Calzolari M (46)	2020	Infected <i>Culex</i> spp., <i>Culex</i> spp. abundance	ND	Lineage 2	Italy	Mosquito abundance, virus presence in mosquitoes	AM	12
21	Benjelloun A (47)	2017	ND	Infected horses	ND	Morocco	Horse cases	AM	10
22	Roiz D (48)	2015	<i>Cx. theileri</i> , <i>Oc. caspius</i> , <i>Cx. modestus</i> , <i>Cx. perexiguus</i> , <i>Cx. pipiens</i> , <i>Oc. detritus</i> presence and abundance	ND	ND	Spain	Mosquito presence and abundance	AM	10
23	Rosà R (49)	2014	<i>Cx. pipiens</i> abundance	ND	ND	Italy	Mosquito presence and abundance	AM	12
24	Mulatti P (50)	2014	<i>Cx. pipiens</i> growth rate and population size	ND	ND	Italy	Mosquito presence and abundance	AM, DM	10
25	Esser HJ (51)	2020	<i>Cx. pipiens</i> , <i>Aedes vexans</i> abundance	Bird abundance, livestock	ND	Netherlands	Introduction & establishment of WNV	SM	12
26	Caneloro L (52)	2020	Infected mosquitoes	Infected birds and horses	ND	Italy	WNV circulation	PM	10
27	Ziegler U (53)	2020	Infected mosquitoes, mosquito parameters	Infected birds, horses, and humans	Lineage 2	Germany	WNV circulation	SM	10
28	Bhowmick S (54)	2020	Mosquito parameters	Bird parameters	ND	Germany	Zoonotic cycle	PM	10
29	Mouatassem TF (55)	2020	<i>Culicidae</i> abundance	ND	ND	Morocco	Mosquito abundance	AM	8
30	García-Bocanegra I (56)	2018	<i>Cx. pipiens</i> , <i>Cx. theileri</i> presence	Infected horses	Lineage 1	Spain	Horse cases	PM	10
31	Andreo V (57)	2017	ND	Infected humans	ND	Greece	Human cases	PM	12

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Table 2 (continued)

ID	First author	Year of publication	Analyzed vector (species)	Analyzed reservoirs & hosts	Genetic lineage	Analyzed countries	WNV indicator	Analytical approach	Study quality
32	Trájer AJ (58)	2017	ND	Infected humans	ND	Greece, Hungary, Israel, Italy, Romania	Human cases	AM	12
33	Semenza JC (59)	2016	ND	Infected humans	ND	Europe	Human cases	AM, TM	12
34	Jian Y (60)	2014	<i>Cx. pipiens</i> abundance	ND	ND	Italy	Mosquito abundance	DM	10
35	Trájer A (61)	2014	<i>Culex</i> spp. presence	Infected humans	ND	Hungary	Mosquito presence; Human cases	PM	10
36	Calistri P (62)	2013	ND	Infected horses	Lineage 1	Morocco	Horse cases	PM	12
37	Rodríguez-Prieto V (63)	2012	<i>Cx. pipiens</i>	Horse density, bird presence	ND	Spain	WNV outbreak suitability	PM	9
38	Roiz D (64)	2012	<i>Cx. pipiens</i> , <i>Cx. hortensis</i> abundance	ND	ND	Italy	Mosquito presence and/or abundance	AM	12
39	Bisanzio D (65)	2011	<i>Ochlerotatus caspius</i> , <i>Cx. pipiens</i> , <i>Cx. modestus</i> abundance	ND	ND	Italy	Mosquito abundance	AM, SM	12
40	Pradier S (66)	2008	ND	Infected horses	ND	France	Horse cases	PM	12
41	Leblond A (67)	2007	ND	Infected horses	ND	France	Horse cases	SM	12
42	Balenghien T (68)	2006	Mosquito presence and abundance, host preferences	ND	ND	France	Mosquito presence and abundance	AM	12
43	Marini G (69)	2020	<i>Cx. pipiens</i> , mosquito parameters, <i>Cx. pipiens</i> abundance	Bird parameters	ND	Italy	WNV human transmission risk, bird cases	AM	10
44	Ippoliti C (70)	2019	<i>Culicidae</i> presence	Infected horses	ND	Italy	Mosquito presence, horse cases	PM	12
45	Marini G (71)	2018	<i>Cx. pipiens</i> abundance, mosquito parameters	Bird parameters, infected humans	ND	Italy	Human transmission risk	DM	12
46	Ben Hassine T (72)	2017	ND	Infected humans	ND	Tunisia	Human cases	PM	12
47	Cotar AI (73)	2016	<i>Culex</i> spp. abundance and infection rate	ND	ND	Romania	Mosquito abundance, virus presence in mosquitoes	AM	12
48	Stilianakis NI (74)	2016	<i>Cx. pipiens</i> presence	Infected humans	ND	Greece	Mosquito presence and abundance	AM, PM	12
49	Pradier S (75)	2014	Mosquito parameters	Infected horses	ND	France	Horse cases	PM	12
50	Roiz D (76)	2014	<i>Cx. pipiens</i> , <i>Cx. modestus</i> , <i>Cx. perexiguus</i> , <i>Cx. theileri</i> , <i>Oc. caspius</i> abundance	ND	ND	Spain	Mosquito abundance	PM	12
51	Conley AK (77)	2014	<i>Cx. pipiens</i> presence	Human population density	ND	Egypt, Lebanon (Middle East and North Africa Region)	Mosquito presence	AM, PM	12
52	Carrieri M (78)	2014	<i>Cx. pipiens</i> , mosquito parameters abundance	ND	ND	Italy	Mosquito abundance	AM	10
53	Mughini-Gras L (79)	2014	<i>Ae. albopictus</i> , <i>Cx. pipiens</i> , <i>Oc. caspius</i> , other (total: 13 species) presence	Swine, equine, ruminant and poultry population density, human population density	ND	Italy	Mosquito presence and abundance	AM, PM	12
54	Ponçon N (80)	2007	<i>Cx. pipiens</i> , <i>Cx. modestus</i> , <i>Oc. caspius</i> abundance	ND	ND	France	Mosquito presence and abundance	AM	12
55	Marcantonio M (81)	2015	ND	Infected humans	ND	EU, Albania, Algeria, Israel, Tunisia	Human cases	AM, PM	12
56	Paz S (82)	2013	ND	Infected humans, and horses, bird parameters	ND	Greece, Israel, Romania, Turkey, Hungary, Italy, Spain, Morocco, Bulgaria	Human, horse cases	AM, PM	12
57	Bravo-Barriga D (83)	2017	<i>Cx. pipiens</i> abundance	Presence of ovine, bovine, caprine, poultry and porcine farms	ND	Spain	Mosquito abundance	AM	12
58	Ferraguti M (19)	2016	Mosquito abundance, mosquito diversity	Human population density	ND	Spain	Mosquito abundance	AM	10
59	Montarsi F (84)	2015	<i>Cx. pipiens</i> , <i>Ae. caspius</i> , <i>Ae. vexans</i> abundance	ND	ND	Italy	Mosquito abundance	AM, PM	10
60	Radrova J (85)	2013	<i>Cx. pipiens</i> , <i>Cx. modestus</i> , <i>Ae. cinereus</i> , <i>Ae. vexans</i> presence	ND	ND	Czech Republic	Mosquito presence	AM	11
61	Zittra C (86)	2016	<i>Cx. pipiens</i> and <i>Cx. torrentium</i> abundance	ND	ND	Austria	Mosquito presence and abundance	AM, PM	10

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Table 2 (continued)

ID	First author	Year of publication	Analyzed vector (species)	Analyzed reservoirs & hosts	Genetic lineage	Analyzed countries	WNV indicator	Analytical approach	Study quality
62	Cailly P (87)	2011	<i>Cx. modestus</i> , <i>Cx. pipiens</i> presence	ND	ND	France	Mosquito presence	PM	10
63	Ziegler U (88)	2019	Mosquito parameters	Infected birds	Lineage 2	Germany	Bird cases	SM	10
64	Muñoz J (89)	2012	<i>Cx. modestus</i> , <i>Cx. perexiguus</i> , <i>Cx. pipiens</i> , <i>Cx. theileri</i> , <i>Oc caspius</i> bloodmeal	Infected humans	ND	Spain	Mosquito bloodmeal	AM, PM	10
65	Leblond A (90)	2005	ND	Infected horses, presence of bovine and poultry farms, bird parameters	ND	France	Horse cases	AM	12

AM: Association/correlation models; DM: Density/abundance models; ND: No data; PM: Predictive models; SM: Spatial models; TM: Transmission models.

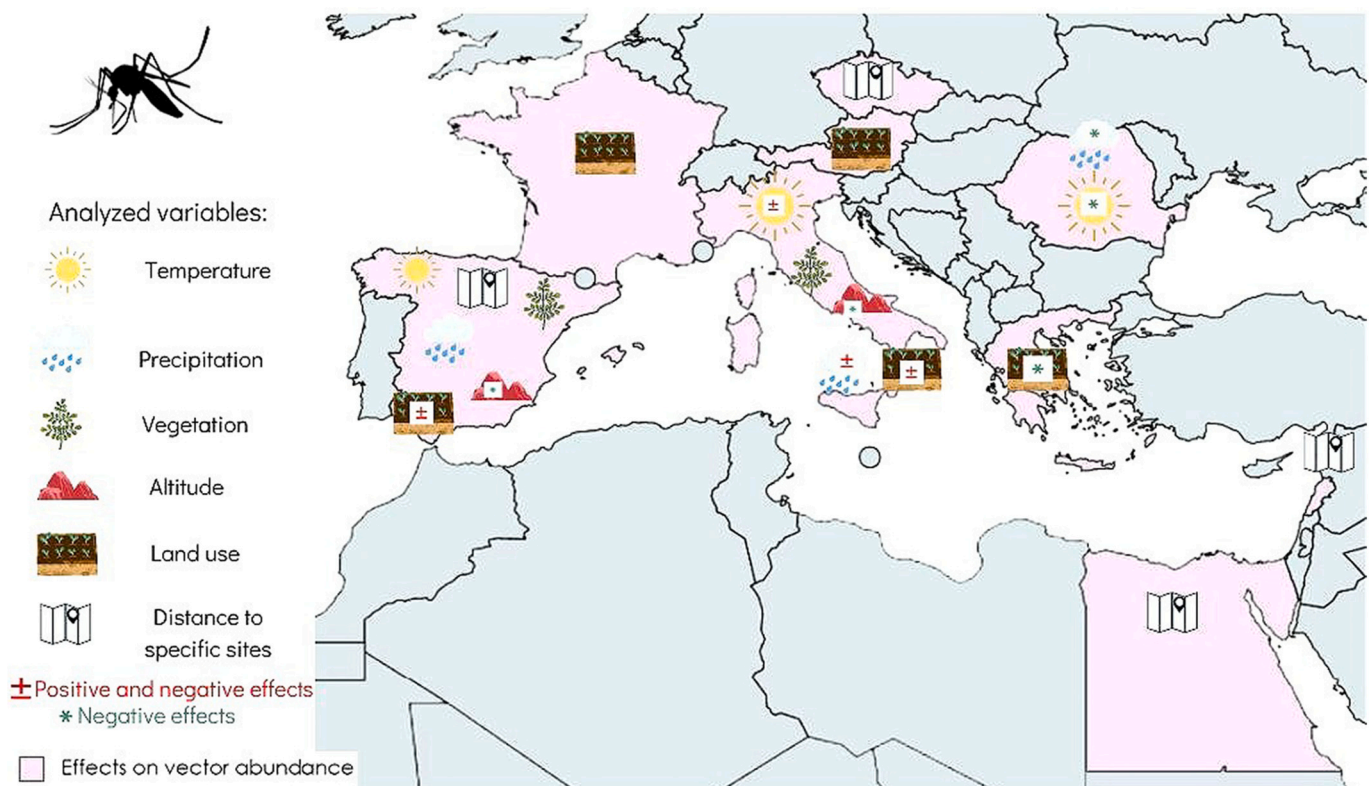


Fig. 3. Significant effects of analyzed environmental variables on WNV vector abundance. Countries with significant relationships between environmental factors and increased WNV vector abundance are shown in violet. The factors significantly related to vector abundance in each country are shown inside the country's shape. Variables with (±) indicate that the effects on vectors were both negative and positive, according to the different studies. Variables (*) indicate that the effects on vectors were negative. Variables with neither (±) nor (*) indicate a positive effect on vectors.

[10,34,35,41,46,47,51,60,63–66,69–72]. Proximity to urban areas showed a positive correlation with *Cx. pipiens* abundance [69]. Distance to aquatic habitats showed a positive correlation for *Cx. pipiens* populations and negative for *Cx. modestus* [71]. In addition, the presence of ovine, bovine and caprine farms within a 500 m radius from a mosquito trapping point [69] and an increased human population density in urban and periurban areas [63] were positive predictors for *Cx. pipiens* abundance.

Regarding land use and land cover [10,17,22,41,46,51,64,65,69,72,73], *Culex* spp. abundance was associated with the expansion of rural [22,64,69], agricultural [10,72], forestal [10], and periurban habitats [69], whereas urban habitat expansion was negatively related to mosquito abundance [10,22,64,69]. However, *Cx. pipiens* is a species particularly well adapted to urban areas and was one of the few species able to maintain significant populations in cities [10,17].

The impact of the NDVI, a proxy for vegetation productivity and biomass on WNV vectors was assessed in relation to vector abundance [10,35,41,46,51], presence [63,65] or both [34], mosquito population growth rates [31] and WNV infection [23]. A positive relationship between NDVI and *Culex* spp. presence [34,63] or abundance [10,31,34,46,51] was reported.

Positive associations were also observed for daylight duration [29,31,36,70,72] and global solar radiation [46] and *Cx. pipiens* population growth rates, i.e., more insulation hours led to larger mosquito populations. On the other hand, high altitude [17,51,69], especially higher than 1700 m was a negative predictor for *Cx. pipiens* [17], *Ae. albopictus*, *Ae. cinereus* and *Cx. modestus* presence [17,65] and *Cx. pipiens* [51,69] and *Cx. modestus* abundance [51].

Other factors that were analyzed were wind [46,60,62,64,70], evapotranspiration [23,62,64], modified normalized difference water

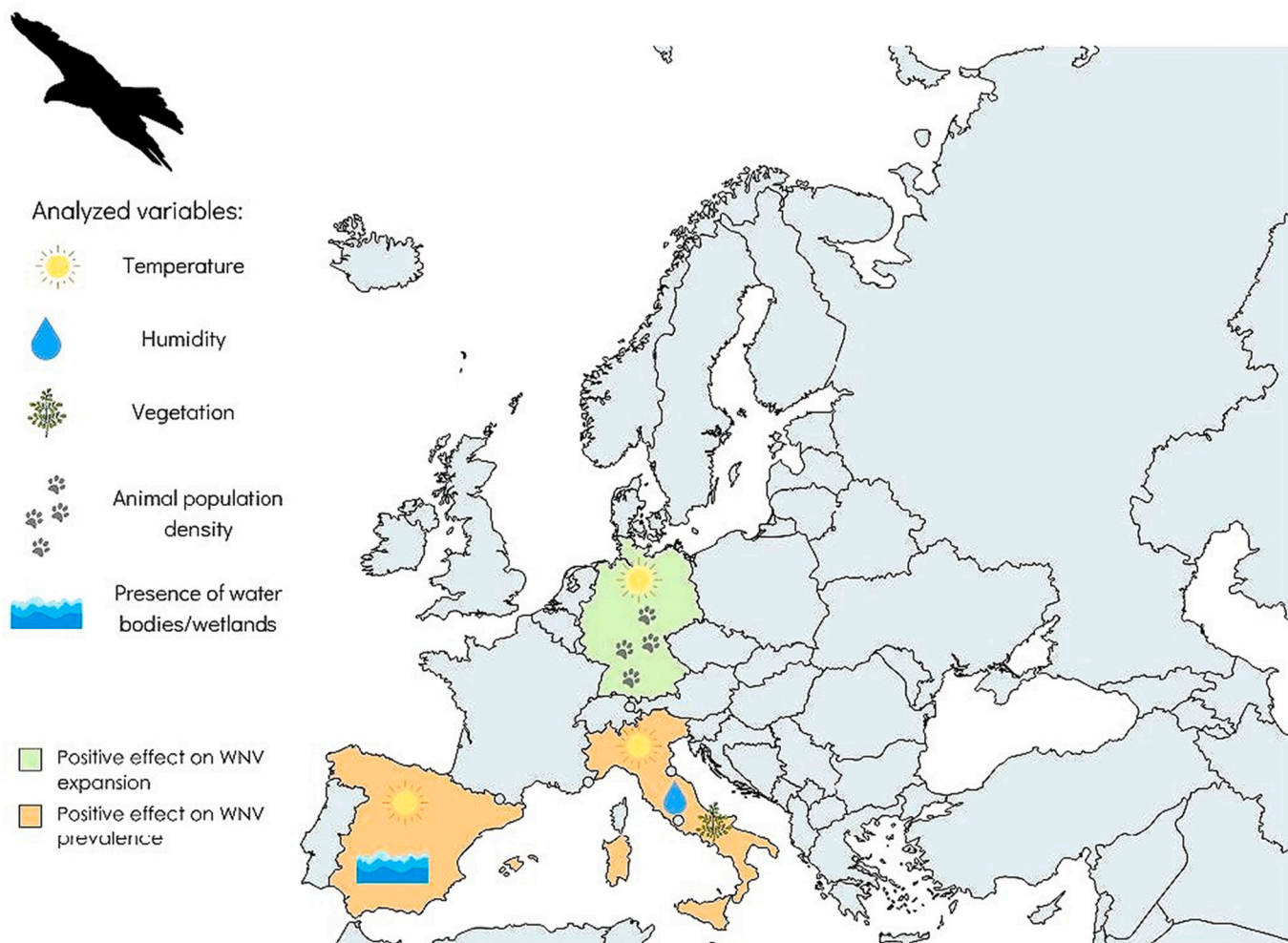


Fig. 4. Effect of analyzed environmental variables on WNV prevalence and incidence in birds. New endemic countries according to the presence of infected birds are shown in green. Countries with increased WNV prevalence in birds are shown in orange. The environmental factors associated with these changes are shown inside the country's shape.

index (MNDWI) [35], tidal data [63] and air pressure [72]. None of these studies observed any impact on vector presence or abundance except for evapotranspiration, which was associated with a higher WNV virus presence in mosquitoes [23]. Fig. 3 shows significant effects of analyzed environmental variables on WNV vector abundance.

3.3. West Nile virus reservoirs

Five studies assessed environmental factors and WNV incidence or prevalence in vertebrate reservoirs (supplementary table 3). Maximum temperatures between June and October and the presence of wetlands in the area were highlighted as positive predictors in predictive risk maps generated from local WNV cases in birds in Southern Spain [15]. Another study observed a positive correlation between WNV circulation in local birds and average temperatures measured between April and June in Northern Italy, mediated by the effect of temperature on vector populations [55]. Yet another study predicted the establishment of WNV in local birds in Northern Germany under future climate change projections for 2017–2100 [40] (Fig. 3).

Finally, one study estimated the risk of WNV circulation in local birds in Italy according to temperature, humidity and NDVI [38], while another study used local bird population density to model and map WNV seropositivity in birds in Germany [74]. In both studies, abnormally high temperatures were related to a greater WNV transmission risk and the authors suggested this was because of decreased extrinsic incubation

period values for WNV in mosquitoes and higher amplification rates, thus infecting more birds [38,74]. Fig. 4 shows the effect of analyzed environmental variables on WNV prevalence or incidence in birds.

3.4. West Nile virus in non-human mammal hosts

Sixteen studies addressed the impact of environmental factors on WNV in non-human mammal hosts (supplementary table 4; Fig. 4). The impact of temperature on the presence of WNV in non-human hosts was the most frequently analyzed relationship [13,15,21,25,33,38,39,42,48,56,68]. Increases in daily land surface temperatures in Israel from 2001 to 2010 were associated with the appearance of more WNV cases in non-human mammals, especially equine cases [13]. In addition, higher daily mean temperatures in July and August in Germany [39] and high spring and summer temperatures in Southern Europe [15,68] were related to a major presence of seropositive horses [13,15,39,68], camels, donkeys, goats, mules, sheep [13].

Six studies highlighted precipitation as a key driver for horse cases [13,33,42,48,56,68]. Increases in mean annual [13,48] and biannual [21] NDVI were also related to a higher number of equine WNV cases in Tunisia [21], Morocco [48] and Israel [13].

The presence of wetlands within a 500 m radius showed a positive association with horse WNV case incidence in France [53,76] and Spain [15]. The risk for WNV appearance in animals was also higher at low altitude areas in Israel [13]. In addition, the proximity to an area

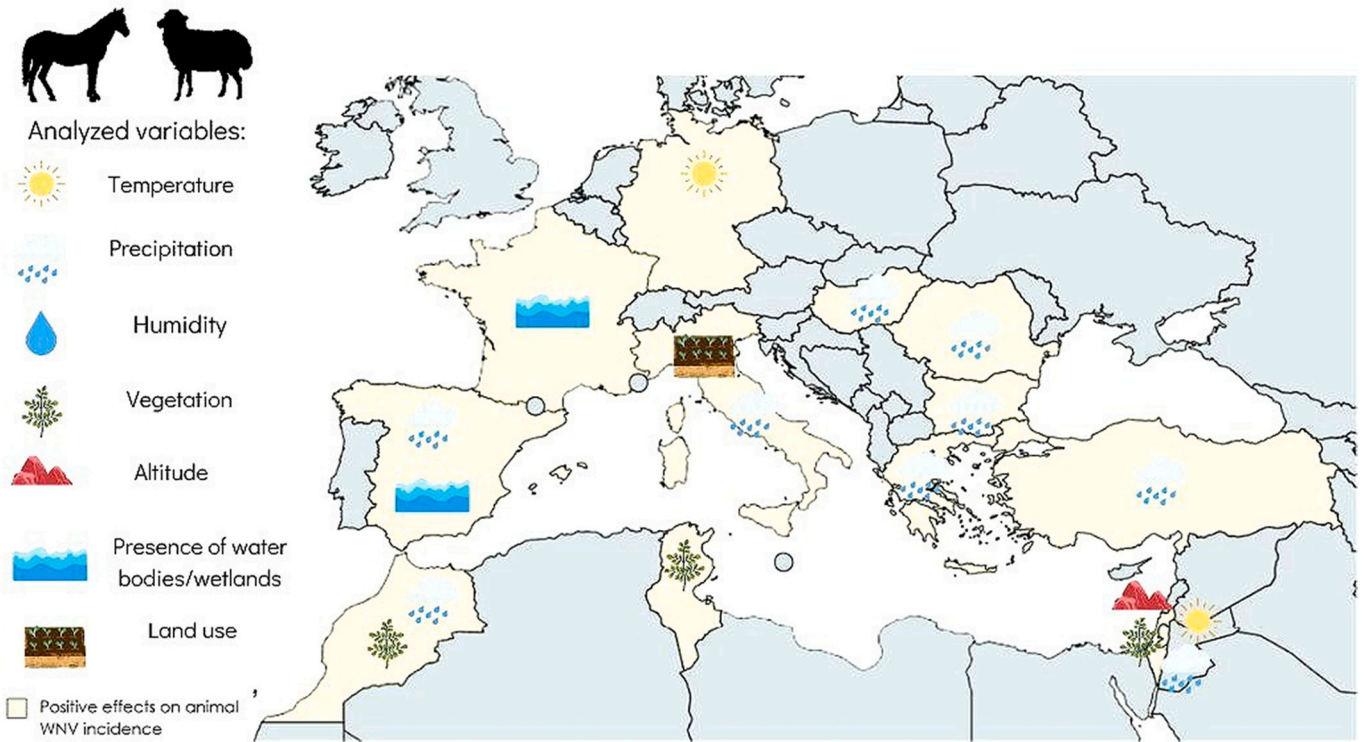


Fig. 5. Effect of analyzed variables on WNV incidence in non-human mammals. Countries with studies analyzing the relationship between environmental factors and WNV incidence are shown in yellow. The environmental factors with significant positive relationships are shown inside the country's shape.

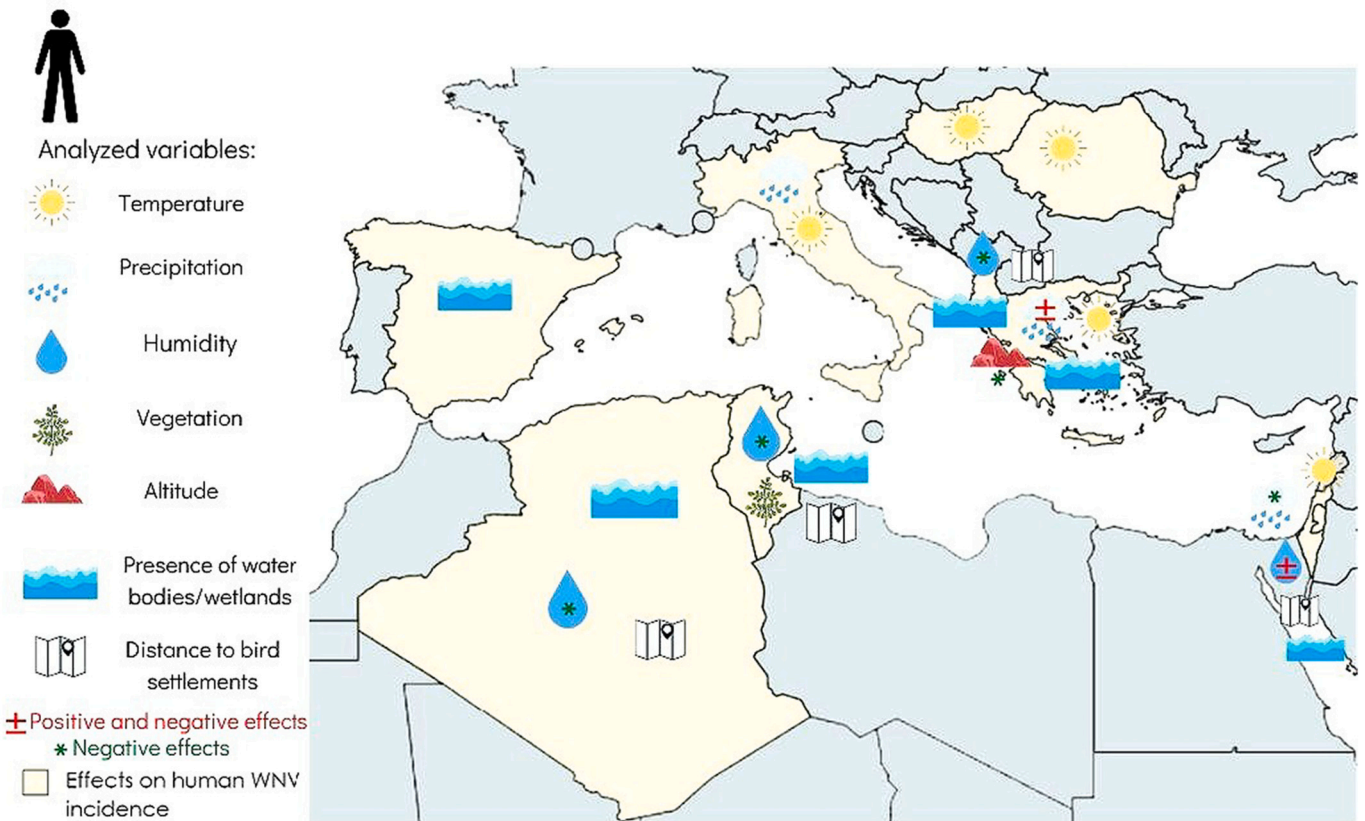


Fig. 6. Relationship between environmental variables and WNV incidence in humans. Countries with reported analyses of relationships between environmental factors and human WNV incidence are shown in yellow. The environmental factors significantly related to WNV incidence in humans are shown inside the country's shape. Variables with (±) indicate that the effects on human hosts were both negative and positive, according to the different studies. Variables with (*) indicate that the effects on human hosts were negative. Variables with neither (±) nor (*) indicate a positive relationship with WNV incidence on human hosts.

suitable for *Culex* spp. showed a positive relationship with the occurrence of equine WNV outbreaks in Italy [65], as well as the presence of mares and breeding sites in France [76]. Fig. 5 shows the effect of analyzed variables on WNV incidence in non-human mammals.

3.5. West Nile virus human hosts

Nineteen studies addressed the relationship between environmental factors and the presence of WNV in humans (supplementary table 5; Fig. 5). Temperature was the most frequently analyzed environmental variable, especially as a predictor for higher human WND incidence [14,15,18,24,27,30,44,45]. Temperature anomalies, i.e., significant deviations during the current season from the average temperatures recorded in the previous years, were positively correlated with the emergence of new human WND cases in different European countries [15,18,27,39,45,47]. Increases of mean and minimum monthly temperatures were related to increased human WND incidence in Israel [24,30], Greece [44] and Italy [16]. Another study observed a higher human WND incidence in Spain in unusually warm summers, which was also related to an increased exposure to WNV vectors during outdoor activities [75]. Seven studies observed no significant relationship between temperature and WND incidence [14,25,33,43,60,67,68].

Precipitation was another predictor for human WND incidence although results were contrasting: two studies observed a positive association between increases in mean and minimum monthly precipitation in Italy [16] and Greece [27], while another observed a negative correlation in Greece and Israel [44]. Five studies observed no relationship between rainfall patterns and WND incidence in humans [14,47,60,67,68].

Another important driver for a higher WND incidence was the existence and/or presence of wetlands in the area [15,18,26,27,45,67]. Similarly, the proximity to bird migration routes was another positive predictor for human WND incidence across the EU [18,45,67]. In another study this relationship with WND incidence was modulated by human population density: at greater density, a greater incidence of human cases was observed [18].

Increased annual NDVI and increased relative humidity also led to a higher human WNV incidence [18,24,26,27], while some negative predictors were altitude, i.e., the lower the altitude, the higher the WNV risk [27]; and the heterogeneity of land use, probably because of smaller vector populations and lower availability of breeding sites [67]. Fig. 6 shows the relationship between environmental variables and WNV incidence in humans.

3.6. Drivers of WNV zoonotic cycle

Two studies focused on both human and other vertebrates WNV cases [25,68], two on WNV in *Cx. pipiens* and hosts [55,56], and six on the complete zoonotic cycle [15,37–40,49].

In one study carried out in The Netherlands, daily temperatures in spring and summer were positively related with WNV establishment, i.e., new detection in vectors and wild birds in previously naïve areas [37]. Mean monthly temperatures were also related to outbreak probability, i.e., if temperature rose, the risk of outbreaks in reservoirs and hosts increased because of better conditions for *Cx. pipiens*, i.e., vector abundance grew [49]. Increased mean temperatures also positively influenced all elements of the zoonotic cycle, especially vectors and reservoirs and, therefore, increased the spread of WNV [40].

The presence of wetlands within a 500 m radius was a positive predictor of WNV circulation in vectors, reservoirs, and hosts in Spain [15].

3.7. Quality assessment

Overall, the studies were of medium or good quality (average 11,1 points). The main reasons for scoring lower were not properly

identifying the sources for data or the study period (supplementary fig. 1). The most frequent limitations identified by the studies' authors were concerns about the study and/or model accuracy ($n = 22$) and the limited data availability ($n = 12$).

4. Discussion

We observed that different environmental factors, such as changes in temperature and rainfall and different patterns of land use, influence the epidemiology of WNV in several countries in the EU and MR [10,13–76]. These changes affect different elements of the WNV zoonotic cycle, with most studies focusing on vectors [10,15,17,22,23,28–32,34–41,46,47,49–51,54–57,59,60,62–66,69–73,75] and hosts [13–15,19–21,24–28,33,37–39,42–45,48,49,52,53,58,60,61,65,67,68,76]. In fact, several studies used these variables as key drivers to model WNV dynamics without analyzing potential correlations between them [14,17,18,20–22,25,27,29,33,38,39,42,43,47,48,52,56,58,60–63,65,67,68,73,75]. Although some studies have analyzed the impact of climatologic factors on WNV in Europe [78], to our knowledge, this is the first comprehensive assessment of the impact of these factors on WNV expansion in the EU and the MR.

Most of the included papers were published during the second half of the study's timeframe, This may be due to rising awareness and interest in WNV, since both human and animal cases are increasing in the EU and MR [4].

Most studies were carried out in Italy [16,22,23,29,31,32,35,36,38,44,46,50,51,55–57,64,65,67,68,70], Spain [10,15,17,34,42,49,62,67–69,75], France [22,52–54,61,66,67,73,76] and Israel [13,19,24,28,30,44,67,68], whereas no studies were performed in certain countries where cases of WNV or an increased seroprevalence have been reported [78–80]. Reasons for this might be the difficulty of performing epidemiological surveillance, particularly vector and animal surveillance at national level, or studies in conflict-affected or politically unstable settings, such as Libya or Syria [81]. Other possible explanations are the lack of funding and/or research interest [82] or a low risk perception [83]. On the other hand, some countries might be over-represented. This does not necessarily indicate a higher WNV incidence in these areas, but points toward a possible publication bias. The first major WNV outbreak among humans in the EU occurred in 1996 in Romania and since then cases and outbreaks have been reported annually, mainly in southern and eastern Europe, especially since the emergence of lineage 2 viruses. A major increase in WND cases was reported in 2018, especially in Italy and Greece [84]. Moreover, it would be interesting for future investigations to consider certain areas that are geographically defined and that share common environmental factors rather than geopolitical divisions; and to conduct prevalence and seroprevalence studies in all the cycle components [vectors, reservoirs, and hosts] for proper risk assessment.

In addition, to evaluate the impact of environmental factors on the spread of WNV, it is of great interest to characterize the lineage of the virus for a comprehensive approach to current and future WNV expansion and to allow more detailed analyses of possible differences in the dynamics of both lineages. This information was only reported in six of the included papers, and both lineage 1 [17,42,48] and 2 [20,39,74] were observed. Whereas lineage 1 has been responsible for several outbreaks in the MR in the last decades, lineage 2 mainly occurred in Sub-Saharan Africa and is now considered endemic in the EU [85].

WNV vectors [10,15,17,22,23,28,29,31,32,34–36,38,41,46,47,50,51,54,55,57,59,60,62–66,69–73,75] and both animal [13,15,19,21,25,33,38,39,42,48,52,53,56,61,68,76] and human hosts [14–16,18,20,24–27,30,39,43–45,47,58,60,67,68,75] were analyzed in most of the studies. In contrast, only five studies addressed WNV reservoirs [15,38,40,55,74]. *Culex* spp. mosquitoes were the most frequently analyzed vectors [15,17,22,23,28,29,31,32,34–37,41,46,47,49–51,56,57,59,60,63,64,73,75], while other potential vectors, especially *Aedes* spp., were analyzed less frequently [86]. The

different vector species show different affinity for different hosts. Some *Culex* spp., specifically *Cx. pipiens*, *Cx. modestus* and *Cx. perexiguus*, prefer to bite birds, whereas *Aedes* spp. and *Cx. theileri* are more anthropophilic and prefer to feed on mammals and *Cx. hortensis* feeds exclusively on reptiles [50]. *Culex* species with a predominantly ornithophilic diet have been pointed out as the main amplifiers and/or bridge vectors of WNV to humans [50,75]. In addition, each species can show a different behavior in relation to environmental factors [62]. Studies focusing on animal hosts were mainly performed on horses, which act as the main non-human accidental WNV hosts. An equine vaccine against WNV has been developed and is being administered to horses [87] and consequently, more studies focusing on the main reservoirs are necessary to understand the influence of the environment on WNV transmission.

Most analyzed papers showed that WNV vector populations, both their presence and abundance, were positively affected by increases in temperature [15,22,23,28,29,29,32,35,36,46,50,54,55,59,62,64–66,69,70,72], precipitation [31,35,36,50,59,64–66,72], NDVI [10,31,34,46,51,63] and the expansion of anthropized areas [10,22,64,69,72]. Studies performed in other regions like the United States of America or Singapore showed similar findings, especially regarding temperature and precipitation [87,88]. Most studies addressed *Culex* spp., especially *Cx. pipiens*, although *Cx. theileri*, *Cx. modestus* and *Cx. perexiguus* were also considered [15,17,22,23,28,29,31,32,34–37,41,46,47,49–51,56,57,59,60,63,64,73,75], which is a result of *Culex* spp. being particularly abundant in the studied regions [89]. Increases in temperature parameters were associated with increases in vector populations [15,22,23,28,29,29,32,35,36,46,50,54,55,59,62,64–66,69,70,72], although a few studies also found a non-linear relationship with mosquito abundance also decreasing when temperature is very high [62]. This can be due to the negative effect that extremely high temperatures may have on different mosquito life-history parameters [9]. However, a delayed summer season was related to a shorter vector season [35,64]. This might be due to *Cx. pipiens* optimal colony rearing temperatures lying between 14 °C and 34 °C [90], a temperature range which is usually reached in late spring to early summer in Europe. Similarly, if the rainy season was delayed 10 to 30 days, mosquito populations decreased, and the vectors' season shortened [50,59]. In these papers, this decrease was explained by the dilution of larvae nutrients in the wettened soil [50,59]. Interestingly, distance to aquatic habitats showed a positive correlation for *Cx. pipiens* populations and negative for *Cx. modestus* [71]. *Cx. pipiens* females usually lay their eggs on water surfaces having low salinity in comparison to other mosquito species, whereas *Cx. modestus* shows higher tolerance toward slightly salty waters [90].

Our review shows that most studies focused on either vectors or human or animal hosts, whereas only five studies addressed reservoirs [15,38,40,55,74]. Birds play an important role in the spread and transmission of WNV in different regions, not only because they act as reservoirs, but because of their migratory behavior [91]. European wetlands where large bird populations are found, are at risk of WNV establishment and circulation [92]. Increases in temperature not only increase vector populations up to a certain level, but also alter bird distribution and habitat use directly and indirectly [93]. Droughts may also alter bird distribution. If birds concentrate on remaining flooded areas where competent WNV vectors and hosts are present, WNV amplification may be facilitated [94]. In addition, anthropogenic changes in habitat might produce a shift toward areas close to human or cattle settlements, thus also increasing the risk of WNV transmission [95]. Landscape changes due to agriculture and human settlements in previously uninhabited areas not only affect different avian populations, but they also contribute to affect bird species' behavior and season-specific movements [96]. Post reproductive short and medium distance movements of birds may also contribute to virus dispersal, due to the time overlap with the period of more intense WNV circulation in

Europe. However, although bird movements and migrations are repeatedly mentioned in WNV literature, studies dealing with the importance of bird movements and population dynamics on WNV transmission are scarce [97].

Increases in temperature [13,15,39,68], vegetation indexes [13,21,48], precipitation [19] and short distances to water bodies [15,53,76] resulted in more WND animal cases, mainly due to the same causes as for human hosts: increased exposure to vectors. In addition, as agricultural areas increase, the presence of competent WNV vectors lead to increased WND incidence [98]. However, to assess the real impact of environmental factors on exposure of cattle to WNV, it would be interesting to include how many animals are at risk of contracting the disease in the analyses of WND incidence, because this information is missing in most studies.

As for humans, increased temperature [16,18,24,30,44,45,75] and short distances to both water bodies [15,18,26,27,45,67] and important areas for bird conservation [18,45,67] were related to an increased human WND incidence, probably because of increased exposure to WNV vectors or increased infection rate in competent vectors. Similar studies which were performed in other regions and/or on other arboviruses showed similar results [93,95]. Moreover, warmer temperatures not only affect mosquito population growth rates, but also human behavior, with a shift toward outdoor activities and, consequently, increased exposure to vectors [95]. This, combined with environmental anthropogenic changes, such as increased agricultural areas or settlements in close contact with nature and animals, leads to increased risk of WNV transmission to humans [98].

In this review we have grouped studies according to the part of the zoonotic cycle they addressed. Difficulties arise when analyzing these parts separately instead of as a complete cycle. This partition is fictional and in reality, all parts intercorrelate [99]. None of the papers reviewed in this study analyzed WNV from a One Health approach including the whole zoonotic cycle. For the control of zoonotic diseases, it is particularly interesting to address all aspects of the disease, i.e., vectors, reservoirs, and hosts, adopting a holistic and comprehensive approach that should be favored in future studies.

Many changes in environmental factors are related to climate change, which is defined as a long-term shift in climate patterns. Impacts of a changing climate are not uniformly distributed across all regions [100]. Consequently, although we can expect the distribution of *Cx. pipiens* to expand in northern areas of Europe due to increases in temperature and thus, in habitat suitability, in some areas of the southern range we expect their populations to remain stable [62] or even to decline [17]. But the impact of climate change will not only affect vector populations but may also have important impacts on vector life history traits and virus development that may increase viral amplification under some temperature ranges or decrease it over some thresholds [9]. Therefore, a comprehensive approach including possible transmission areas rather than only geopolitical frontiers might contribute to improve existing evidence on the impact of climate change on WNV.

Our study has several shortcomings. First, we performed a search that was bound to certain inclusion criteria and a specific search strategy, which could have led to the non-inclusion of all relevant articles. In addition, selection bias might also affect our study. However, we have followed the PRISMA guidelines for systematic reviews to limit this bias. We limited our search to articles published in the included languages. The language criteria exclude articles published in languages spoken in EU/EEA and MR countries. However, since all included articles were published in English, we believe that most relevant articles were included. Lastly, the included articles were of different methodological quality. To cope with this limitation, we used a specific tool to evaluate the studies' quality, which on averaged scored very high.

5. Conclusion

WNV has currently expanded to several countries in the EU and MR.

The environmental factors that most frequently correlated to changes in WNV dynamics were temperature, precipitation, vegetation and the presence of water bodies or different types of land use, such as agricultural or rural environments. Most studies observed a positive relationship, although urban habitats were associated to a decreased human WNV incidence. Since we addressed all elements of the zoonotic cycle and the impact of different environmental factors, we believe that this study can help researchers to select more correctly what needs to be analyzed and to detect current research gaps to provide a comprehensive understanding of WNV in the EU and MR. In this sense, we found that most studies focused on WNV vectors and hosts, whereas only few addressed bird reservoirs. It is important to highlight that the relationships between environment and WNV do not need to be linear, although this possibility has been less investigated. In addition, we hope that the results of this study translate into concrete public health measures to tackle WNV expansion in the EU and MR. Since our analysis relies on the results of published studies whose average quality was good, our results are reliable and provide a comprehensive review of different environmental factors and WNV in vectors, reservoirs and hosts in EU and MR countries. It also needs to be clarified whether increased WNV expansion is a result of warming climate and if current climate change knowledge enables us to predict future WNV expansion. Finally, different WNV lineages are occurring in Europe, but little is known on the consequences of environmental changes on the transmission of each one of these lineages. It needs to be clarified whether the increased expansion of lineage 2 in Europe and apparent decline of lineage 1 are a consequence of environmental factors favoring lineage 2. Therefore, we believe that a comprehensive One Health and transnational approach would be suitable and necessary to analyze and predict current and future WNV epidemiology.

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Declaration of Competing Interest

None.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.onehlt.2022.100478>.

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