Case-Time Series Study on the Short-term Impact of Meteorological Factors on West Nile Virus Incidence in Italy at the Local Administrative Unit Level, 2012 to 2021.

Luigi De Angelis, Angela Ancona, Giovenale Moirano, Aurea Oradini-Alacreu, Antonino Bella, Massimo Fabiani, Daniele Petrone, Emanuela Piervitali, Walter Perconti, Piero Fraschetti, Giulio Settanta, Martina Del Manso, Emmanouil Alexandros Fotakis, Flavia Riccardo, Caterina Rizzo, Patrizio Pezzotti, Alberto Mateo-Urdiales

PII: S0013-9351(24)02227-8

DOI: https://doi.org/10.1016/j.envres.2024.120320

Reference: YENRS 120320

To appear in: *Environmental Research*

Received Date: 10 September 2024

Revised Date: 21 October 2024

Accepted Date: 6 November 2024

Please cite this article as: De Angelis, L., Ancona, A., Moirano, G., Oradini-Alacreu, A., Bella, A., Fabiani, M., Petrone, D., Piervitali, E., Perconti, W., Fraschetti, P., Settanta, G., Del Manso, M., Fotakis, E.A., Riccardo, F., Rizzo, C., Pezzotti, P., Mateo-Urdiales, A., Case-Time Series Study on the Short-term Impact of Meteorological Factors on West Nile Virus Incidence in Italy at the Local Administrative Unit Level, 2012 to 2021., *Environmental Research*, https://doi.org/10.1016/j.envres.2024.120320.

Title: Case-Time Series Study on the Short-term Impact of Meteorological Factors on West Nile Virus Incidence in Italy at the Local Administrative Unit Level, 2012 to 2021.

Authors: Luigi De Angelis (https://orcid.org/0009-0005-6136-6596)[1,2], Angela Ancona[1,3], Giovenale Moirano[4,5], Aurea Oradini-Alacreu[1,3], Antonino Bella[1], Massimo Fabiani[1], Daniele Petrone (https://orcid.org/0000-0001-8193-5446) [1,6], Emanuela Piervitali[7], Walter Perconti[7], Piero Fraschetti[7], Giulio Settanta[7], Martina Del Manso[1], Emmanouil Alexandros Fotakis[1,8], Flavia Riccardo[1], Caterina Rizzo[2] (https://orcid.org/0000-0002-5583-7508), Patrizio Pezzotti[1](https://orcid.org/0000-0002-0805-2927), Alberto Mateo-Urdiales[1] (https://orcid.org/0000-0002-0480-8723)

Affiliations
1. Department of Infectious Diseases, Istituto Superiore Di Sanità, Rome, Italy
2. Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy
3. School of Public Health, Vita-Salute San Raffaele University, 20132 Milan, Italy.
4. Cancer Epidemiology Unit, Department of Medical Sciences, University of Turin, Turin, Italy
5. Barcelona Supercomputing Center (BSC), Barcelona, Spain
6. Department of Statistics, Sapienza University of Rome, 00185 Rome, Italy.
7. Italian Institute for Environmental Protection and Research (ISPRA), Rome, Italy
8. European Programme On Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control, Stockholm, Sweden

Correspondence: Luigi De Angelis (l.deangelis2@studenti.unipi.it) Via San Zeno 35, IT-56123, Pisa (PI), Italy

Keywords: Climate Change; Vector-borne diseases; West Nile Virus; Case Time Series; Distributed Lag Non Linear Models

Collaborators
None.

Statements

Ethical statement

The data utilized in this study adhere to the ethical guidelines and regulatory frameworks established by Italian health authorities for the surveillance of arboviruses. According to the *Nota ministeriale 013307 of the 18th of May 2023,* the *Circolare Ministero della Salute 0019613* of the *10th of August 2022*, and the *Piano nazionale di prevenzione, sorveglianza e risposta alle Arbovirosi (PNA) 2020-2025* (National Plan for Prevention, Surveillance, and Response to Arboviruses 2020-2025*)* data collection falls under the national surveillance activities, which are exempt from Ethical Committee review. No personal patient data was used without appropriate de-identification to ensure privacy and compliance with applicable laws.

Funding statement
None declared.

Use of artificial intelligence tools
None declared.

Data availability
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Preprint
None.

Acknowledgements
We acknowledge Giorgio Cattani from the Institute for Environmental Protection and Research (ISPRA) for his contribution to collecting meteorological data for our study.

Conflict of interest
None declared.

Authors' contributions
LDA, AA, AMU guided the development of the paper and the epidemiological analysis with the support of PP, GM, AOA, AB, EF. MF, DP, MDM and FR provided input and suggestions towards the development of the paper and validated the analysis. EP, WP, PF, GS coordinated the collection of meteorological data and provided feedback on the methodology. PP, CR and AMU provided expert advice and support in the development of the manuscript. All authors reviewed and approved the final version of the manuscript.

Abstract

Introduction

West Nile Virus (WNV) is a significant public health concern in southern Europe, with meteorological, climatic, and environmental factors playing a critical role in its transmission dynamics. This study aims to assess the short-term effects of meteorological variables on the incidence of WNV in five Italian regions in Northern Italy from 2012 to 2021.

Methods

Linking epidemiological data from the national surveillance system and local meteorological data, we conducted a Case-Time Series analysis to examine the association between WNV incident cases and temperature, humidity, and precipitation recorded up to ten weeks before case occurrence at the local administrative unit level. We employed conditional quasi-Poisson regression and distributed lag non-linear models to explore delayed effects.

Results

Our study analyzed 1,110 autochthonous human cases of WNV. We found a positive association between WNV incidence and weekly mean temperature recorded between one to nine weeks before the diagnosis, with the highest effect at one week lag (IRR: 1.16; 95% CI 1.11-1.21). An increase in weekly precipitations between the sixth and ninth weeks before diagnosis was also positively associated with WNV incidence. Variations in minimum weekly humidity did not show a consistent impact.

Conclusions

Our findings underscore the influence of temperature and, to a lesser extent, precipitation on WNV incidence in Northern Italy, highlighting the potential of climatic data in developing early warning systems for WNV surveillance and public health interventions.

Main Text

1. Introduction

West Nile virus (WNV) is a single-stranded RNA virus belonging to the Flaviviridae family, genus Orthoflavivirus. Human infection is primarily associated with lineages 1 and 2 [1].

The virus was originally isolated in 1937 in Uganda from the blood of a woman with febrile symptoms in the West Nile district. WNV is currently widespread in Africa, the Middle East, North America, Asia, and Europe, where it has been reported since 1958. In Italy, WNV was first isolated in 1998 in horses,[2] while the first autochthonous human case was reported in 2008 in the northern area of the country (Emilia Romagna region)[3]. Currently, WNV is endemic in Northern Italy, with cases reported every year[4].

The WNV vectors are mosquitoes of the genus Culex (Cx.) such as *Cx. pipiens, Cx. tarsalis* and *Cx. Quinquefasciatus*[5,6]. Birds act as amplifying hosts or virus reservoirs[7]. Humans and other mammals are considered dead-end hosts.

In humans, the incubation period ranges between 2 and 14 days but may extend to 21 days in immunocompromised individuals [1] Up to 80% of the infected subjects do not develop clinical symptoms. The remaining 20% show an influenza-like syndrome called West Nile fever (WNF) [8]. Less than 1% of infected subjects develop a neurological syndrome, known as

West Nile Neuroinvasive Disease (WNND), characterized by symptoms such as meningitis, encephalitis, or acute flaccid paralysis [1].

It is widely accepted that temperature, air humidity, and the amount of precipitation have a significant impact on the circulation of vector-borne viruses, including the WNV, with numerous studies over the last years assessing the impact of these meteorological drivers on the transmission and spread of arboviruses [9,10].

However, the relationship between meteorological factors, environmental conditions, and the incidence of WNV is extremely complex. Multiple variables have been suggested to explain the spread of WNV observed in Europe in the past years. These variables include climatic factors, such as abundant rainfall in late winter and early spring, high summer temperatures, and summer drought; hydrological factors, such as the presence of wetlands and stagnant water; and phytogeographical factors, such as a high percentage of land with irrigated fields and forest environments [9].

Climate change is further increasing the complexity, as it may have a direct effect on vectors dynamics as well as on the ecosystems in which vectors and hosts live. Moreover, it is also possible that climate change is altering the migratory birds' behaviour, anticipating their arrival in increasingly warmer spring seasons. As a result, it has been suggested that climate change can increase WNV transmission dynamics and geographical distribution through multiple pathways that include direct effects on the virus, the mosquito vector, non-human hosts, and humans [11].

In Northern Italy, West Nile disease (WND) is an endemic-epidemic disease more prevalent from June to October, and three major outbreaks have been reported in 2018, 2022, and 2023 [12,13]. Better characterizing the role of meteorological factors in driving the timing and intensity of past WNV outbreaks in the area might provide useful information to establish early-warning systems. Identifying the best time lag that elapses between meteorological predictors and the outcome of interest can provide useful information for seasonal and sub-seasonal forecasting.

The aim of this study is to evaluate the short-term effects, in the 10 weeks before the diagnosis, of meteorological variables on the incidence of human cases of WNV infection at the municipality level in Northern Italy from 2012 to 2021.

2. Methods

2.1 Data Collection

For this study, we used epidemiological data of human cases of confirmed WNV infection reported to the national surveillance system in Italy from 2012 to 2021. This surveillance system is coordinated by the Italian National Institute of Public Health (ISS) and uses the case definition of human WNV proposed by the EU Commission Implementing Decision 2018/945 [14]. The surveillance system collects individual data on WNV cases, including asymptomatic individuals usually identified through blood donation screenings, WNF, and WNND cases at the local administrative unit level, which in Italy corresponds to municipalities [4]. The surveillance system also collects clinical and demographic data, including the municipality of

probable infection. We selected autochthonous cases from five Italian regions: Veneto, Lombardia, Emilia Romagna, Piemonte, and Friuli-Venezia Giulia. These regions were chosen because they are the most affected areas and have the most established surveillance systems for monitoring WNV [15].
For each municipality reporting WNV cases in the study period, weekly time series of WNV cases were computed aggregating the cases by the week of symptoms onset for WNF and WNND and the week of laboratory diagnosis for asymptomatic cases.

Meteorological data were provided by the Italian Institute for Environmental Protection and Research (ISPRA), detailing daily records of maximum, minimum, and average temperatures and humidity, precipitation, and maximum, minimum, and average humidity for each municipality.[16] Data obtained from ISPRA were recorded by land-based meteorological stations but many data points were missing (64%, 710/1,110). If meteorological data for a certain municipality were missing, we considered data from the closest weather station, assuming the same meteorological conditions in close areas. The median distance to the closest station was 20.1 km (IQR: 5.1- 49.7 km), distance was considered zero for adjacent municipalities. For each municipality included in the study we computed weekly averages for temperature and humidity variables and weekly cumulative precipitation.

Demographic data on the total inhabitants and urbanization level of each municipality were obtained from the estimates produced by the Italian National Institute of Statistics (ISTAT) [17].

2.2 Study Design

We applied a Case Time Series (CTS) design to investigate the relationship between meteorological variables and WNV incident cases. The CTS design is based on the definition of observational units for which exposures and health outcome data are longitudinally collected. In this study, municipalities were defined as observational units, with weekly WNV cases representing the health outcomes and weekly series of meteorological factors lagged up to 10 weeks representing the exposures of interest. For each observational unit, matched risk sets can be defined for different spatio-temporal strata, thus modelling within-stratum variations in risk. In our study, strata were defined by the combination of municipality, year and month of symptoms onset or diagnosis. Municipalities were included in the study only if they reported at least one autochthonous WNV case during the study period. Analysis was restricted to strata with reported cases in a specific month, year, and municipality, effectively excluding months with no cases. This approach ensures that all analyzed data are relevant to periods of active WNV transmission in the area allowing to identify the triggering effect of meteorological variables on the occurrence of WNV cases. The CTS design focuses on comparing periods that are close in time (e.g., within the same month), allowing for an evaluation of short-term effects. By design, months without any cases do not allow for meaningful comparison. CTS is suitable for analyzing time-varying exposures allowing for the control of seasonal and geographical confounding as well as for unmeasured confounders by design [18,19], as in other self-matched methods [20]. To analyze the short-term effect of climatic variables we considered 10 weeks of lag and excluded lag 0, based on the WNV incubation period [21].

2.3 Statistical Analysis

The analysis was performed using a conditional quasi-Poisson model to manage overdispersion in our data, exploring the influence of weather conditions in the 10 weeks preceding WNV symptoms onset or diagnosis. We employed a distributed lag non-linear model to assess the delayed effects of meteorological factors on WNV cases (DLNM). DLNM are two-dimensional models developed to explore exposure-lag-response relationships along both the dimensions of exposure and lag [22]. In our study, the effect of climatic parameters was modeled with a linear function, while the lag effect was modeled through a cubic basis spline with 4 degrees of freedom, as in a previous study [23]. The model incorporated the logarithm of the population as an offset to calculate lag-specific incidence risk ratios (IRRs) for unit increase in temperature, humidity, and precipitation. The unit was set as 1 °C for the weekly average of temperatures, 3% for the weekly average of humidity, and 10 mm for the weekly total precipitation. In addition, we estimated the overall cumulative effect, that is the sum of each specific lag contribution over the whole lag period and can be interpreted as the overall risk. Univariable and multivariable analyses were conducted for each of the meteorological variables. A lower quasi-AIC in the univariable analysis was chosen as the criteria for selecting which meteorological variable among minimum, maximum, and mean temperature and humidity to include in the multivariable analysis. We assessed the possible presence of exteremely high correlation among variables included in our multivariable analysis through the adjusted Generalized Variance Inflation Factor (GVIF), finding no evidence of multicollinearity (all adjusted GVIF<2).The sameanalyses were conducted on WNND cases only, as surveillance activities are considered more reliable in this subset of cases. The software used was R, version 4.3.1 (2023). Packages used included GNM[24] and DLNM[25].

3. Results

3.1 Descriptive Results

From 2012 to 2021, 1,110 autochthonous human cases of WNV infection were reported to the national surveillance system in the five selected Italian regions of this study. The year with the highest number of cases was 2018, accounting for 54% (n=600) of the total cases. These cases were reported between the 24th and the 42nd week of the year with a median of 51 weekly cases (IQR: 4.5 - 105), with 98% of the infections occurring from weeks 28 to 39 (n = 1,089). Weeks 32, 33, and 34 saw the peak in diagnosis, coinciding with August.

Among the five regions analyzed, Veneto was the most affected, recording 37.6% (n=417) of the total reported cases, followed by Emilia-Romagna with 33.1% (n=368). Lombardia, on the other hand, reported 18.1% (n=201) of the total infections, Piemonte 7.5% (n=83) of the cases, and Friuli-Venezia Giulia 3.7% (n=41) of the cases. WNV in Veneto and Friuli-Venezia Giulia was already reported in 2012, while Emilia-Romagna and Lombardia reported WNV cases starting in 2013 and Piemonte in 2015. Maps of yearly incidence rates for each municipality are reported as Supplementary Materials (S1). The cumulative incidence of WNV between 2012 and 2021 in Northern Italy was respectively 4-fold and 10-fold higher in suburban and rural areas as compared to urban areas.

As reported in Figure 1, of the 1,110 cases reported, 46.9% (n=520) were cases of neuro-invasive disease, followed by 38.5% (n=428) cases of WNF, and 14.6% (n=162) asymptomatic cases.

Across the study period, 541 municipalities reported at least one human case of WNV and were therefore included in the analysis. When stratifying by municipality(541), years(10), and months(12), 3904 strata out of 64,920 (1.38%) presented at least one case of WNV and were therefore included in the analysis. The overall cumulative incidence for each municipality across the study period was calculated as the total number of WNV cases per 10,000 inhabitants. The affected municipalities show a pattern that geographically coincides with the Po Valley. Figure 2 shows cumulative incidence rates in the period of study by municipality.

3.2 Univariable and Multivariable Results

In the univariable analysis, the mean temperature and the minimum humidity had the lowest qAIC and were, thus, included in the multivariable analysis. Results of the univariable analyses are reported as Supplementary Materials (S2). Table 1 presents the lag-specific Incidence Rate Ratios (IRRs) resulting from the multivariable analysis.

	1°C increase in weekly mean temperature	**3 % increase in weekly mean minimum humidity**	**10 mm increase in weekly cumulative precipitations**
Lag (weeks)	**IRR (95%CI)**	**IRR (95%CI)**	**IRR (95%CI)**
1	1.16 (1.11-1.21)	1.05 (0.99-1.10)	0.98 (0.94-1.03)
2	1.13 (1.10-1.16)	1.00 (0.95-1.05)	1.00 (0.95-1.04)
3	1.10 (1.07-1.14)	0.97 (0.92-1.02)	1.01 (0.96-1.06)
4	1.08 (1.05-1.12)	0.95 (0.89-1.00)	1.03 (0.97-1.09)
5	1.07 (1.04-1.10)	0.95 (0.89-1.00)	1.05 (0.99-1.11)
6	1.06 (1.03-1.09)	0.96 (0.90-1.01)	1.07 (1.02-1.13)
7	1.05 (1.02-1.09)	0.97(0.92-1.03)	1.08 (1.03-1.14)
8	1.05 (1.02-1.08)	0.99 (0.94-1.04)	1.08 (1.02-1.13)
9	1.04 (1.01-1.06)	1.00 (0.96-1.05)	1.05 (1.01-1.10)
10	1.03 (0.99-1.06)	1.02 (0.97-1.06)	1.03 (0.98-1.08)
Cumulative effect	2.09 (1.73-2.55)	0.85 (0.57-1.26)	1.45 (0.99-2.14)

Table 1: Incidence Risk Ratios (IRRs) and 95% Confidence Intervals (CIs) as results of the multivariable analysis including three climatic variables (mean temperature, minimum humidity, precipitations) and the logarithmic of the population as offset. The outcome of the analysis is the incidence of WND cases. IRRs and CIs are reported for individual weekly lags and as a cumulative effect.

Both univariable and multivariable analyses provided a positive relationship between mean temperature and increase in WNV incidence at weekly lags 1-9. The strength of the association between mean temperature and WNV incidence decreases with longer lags reaching the null value at lag 10. The multivariable cumulative IRR of temperature on WND incidence was equal to 2.09, 95%CI 1.73-2.55.

In the univariable analysis, minimum humidity was inversely associated with WNV incidence from lag 1 to 9. However, in the multivariable analysis, increases of 3% in the minimum humidity were not strongly associated with WNV incidence at any lag.

The effect of precipitation on WNV incidence changed between univariable and multivariable analyses. In the univariable analysis, an inverse relationship between precipitation levels and WNV incidence was identified from the second to the fifth week and for the cumulative effect. In contrast, the multivariable analysis provided a positive association between precipitation at lag 6-9 weeks and WND incidence. The multivariable cumulative IRR for precipitation was equal to 1.45, 95% CI 0.99-2.14.

Figure 3 provides information on the trends of association between climatic variables and WNV incidence at increasing lags. Mean temperature has a linear downward trend, while minimum humidity and precipitation have U-shaped and inverted U-shaped patterns fluctuating around the reference line.

Similar results were found repeating the analysesonly on the subset of WNND cases. (Supplementary S3, S4, S5, S6)

4. Discussion

Our investigation into WNV dynamics in Northern Italy highlights a distinct seasonal pattern, with incidence peaks during the summer months. Notably, the magnitude of these peaks varies significantly across years, as exemplified by the high incidence observed in 2018. Certain municipalities near endemic areas in the Po Valley have not reported WNV cases over a decade, suggesting underlying factors that merit further exploration.

In our study, we identified a positive association between temperature over the preceding ten weeks and the incidence of WNV. This relationship was substantiated through both univariable and multivariable analyses, exhibiting consistent trends and magnitudes of association. Our results indicate that minimum humidity alone does not significantly influence WNV incidence when controlling for other climatic factors, confirming evidence from previous studies[26]. Additionally, the univariable analysis results for precipitation were refuted by the multivariable analysis, underscoring the nuanced impact of rainfall on WNV transmission dynamics.The similar results observed anayzing only WNND cases, that are more likely to be diagnosed, exclude the potential biases introduced by differences in surveillance effort.

Consistent with existing literature, our findings indicate that higher temperatures are associated with increased WNV incidence at short lags.[27,28] These findings are reinforced by previous studies documenting that temperature is a driver of WNV transmission, influencing vector lifecycle and virus replication rates.[29,30] For instance, in the temperate zones of southern Europe, mosquito activity is at its peak between 22 and

30°C [11]. This is also supported by entomological data showing that the capture of *Cx. pipiens* significantly rise if there is an increase in average temperatures in the previous 11 days.[31] Elevated ambient temperatures increase the growth rate of the vector population, decrease the interval between blood meals, and reduce the extrinsic incubation period.[32] Temperature and other climatic variables not only influence mosquito life cycles but also have a multifaceted impact on WNV incidence, for instance, higher temperatures often encourage people to spend more time outdoors, which increases their exposure to mosquito bites.

The impact of precipitation and humidity on WNV seasonality remains a topic of debate within the scientific community.[33,34] While some research indicates that delayed rainfall onset extends the WNV transmission season,[35] other studies suggest that mosquito populations—and by extension, WNV incidence—are higher in drier conditions.[3][36]Our analysis suggests that understanding the relation between precipitation and WNV incidence is complex, as we found a negative association between WNV cases and abundant rainfall in univariable analyses and a positive association in multivariable analyses. This relationship may be attributed to the critical role played by the combination of temperature and precipitation, though the precise mechanisms warrant further investigation.[37]

Comparing our results with a similar study conducted on WNV cases in Northern Italy from 2010 to 2015 reveals some differences in the observed impacts of climatic variables.[23] For instance, the previous study identified a positive association between WNV incidence and temperature recorded between 2 and 5 weeks before the diagnosis, while we observed it already at shorter lags. These disparities could stem from variations in sample size, study design, the spatial resolution of the meteorological data considered, and the rate of the changing climate during the last 15 years.[38]

Our study has several strengths. Methodologically, we carried out a practical implementation of the CTS design recently introduced by Gasparrini et al.[18]. This study design combined with the use of DLNMs allows for a more nuanced understanding of time-varying exposures, particularly relevant in the context of infectious disease epidemiology where environmental conditions play a crucial role. To our knowledge, this is the first study evaluating the role of meteorological variables on WNV transmission using a high spatial resolution by focusing on local administrative units (municipalities). This level of granularity surpasses that of previous studies on the subject, which typically relied on data aggregated at the NUTS3 level (province), as sourced from the European Surveillance System (TESSy).[11,39]

One limitation of our study is the lack of data on WNV lineage, which limited our ability to assess the differential impact of climatic variables on virus transmission dynamics. This is particularly relevant given the co-circulation of WNV lineage 2 and the recently introduced WNV-1a strain identified for the first time in the Veneto region in 2021.[13] Additionally, applying a CTS we could not examine the long-term effects, as seasonal or decadal effects, of climatic variables on WNV transmission. Moreover, there could be unmeasured confounders that could influence WNV dynamics independently of meteorological variables, such as land use changes, vector control efforts, human population movement, and bird migration patterns. For this reason we were not able to create a predictive model for WNV incidence. Lastly, the unavailability of meteorological data for several data points could potentially limit the precision of our analysis. However, we addressed this issue by using data from the nearest meteorological stations when local data were unavailable. This approach, while not ideal, still allows us to achieve a higher spatial resolution compared to many other environmental studies on WNV.

Our research lays the groundwork for the development of an early warning system that could enhance vector and human surveillance by incorporating meteorological data to identify potential spatial hotspots for WNV transmission with a fine spatial resolution. Such a system has the potential to improve public health responses to WNV outbreaks by allowing for more timely and targeted interventions.[40] Leveraging Earth observation data, such as that provided by COPERNICUS,[41] and artificial intelligence,[27] offers a promising avenue for future research in this area. Additionally, a One Health approach should be employed by integrating surveillance data on vectors and animal hosts for accurately predicting WNV circulation.[42] Moreover, expanding the study to multiple locations with varying climate conditions could provide insights into the generalizability of the findings. Finally, integrating climate change projections could offer predictions on future trends in WNV incidence.

5. Conclusions

This study underscores the intricate relationship between meteorological variables and the incidence of WNV in Italy, demonstrating a significant association with temperature in the weeks leading up to diagnosis. The methodology utilized offers a robust framework for investigating the short-term climatic impacts on vector-borne diseases, potentially serving as a foundation for the development of predictive models and early warning systems. Future research should aim to replicate these findings in varied geographical settings to validate the model's applicability and to explore the influence of additional environmental factors on WNV transmission dynamics.

Statements

Ethical statement

The data utilized in this study adhere to the ethical guidelines and regulatory frameworks established by Italian health authorities for the surveillance of arboviruses. According to the *Nota ministeriale 013307 of the 18th of May 2023,* the *Circolare Ministero della Salute 0019613* of the *10th of August 2022*, and the *Piano nazionale di prevenzione, sorveglianza e risposta alle Arbovirosi (PNA) 2020-2025* (National Plan for Prevention, Surveillance, and Response to Arboviruses 2020-2025*)* data collection falls under the national surveillance activities, which are exempt from Ethical Committee review. No personal patient data was used without appropriate de-identification to ensure privacy and compliance with applicable laws.

Funding statement
None declared.

Use of artificial intelligence tools

None declared.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request in compliance with data protection regulations.

Preprint

None.

Acknowledgements

We acknowledge Giorgio Cattani from the Institute for Environmental Protection and Research (ISPRA) for his contribution to collecting meteorological data for our study.

Conflict of interest

None declared.

Authors' contributions

LDA, AA, AMU guided the development of the paper and the epidemiological analysis with the support of PP, GM, AOA, AB, EF. MF, DP, MDM and FR provided input and suggestions towards the development of the paper and validated the analysis. EP, WP, PF, GS coordinated the collection of meteorological data and provided feedback on the methodology. PP, CR and AMU provided expert advice and support in the development of the manuscript. All authors reviewed and approved the final version of the manuscript.

References

1. Petersen LR, Brault AC, Nasci RS. West Nile Virus: Review of the Literature. JAMA [Internet]. 2013 Jul 17 [cited 2024 Mar 27];310(3):308–15. Available from: https://jamanetwork.com/journals/jama/fullarticle/1713596
2. Autorino GL, Battisti A, Deubel V, Ferrari G, Forletta R, Giovannini A, et al. West Nile virus epidemic in horses, Tuscany region, Italy. Emerg Infect Dis [Internet]. 2002 Dec 1 [cited 2024 Mar 27];8(12):1372–8. Available from: https://pubmed.ncbi.nlm.nih.gov/12498650/
3. Marini G, Calzolari M, Angelini P, Bellini R, Bellini S, Bolzoni L, et al. A quantitative comparison of West Nile virus incidence from 2013 to 2018 in Emilia-Romagna, Italy. PLoS Negl Trop Dis [Internet]. 2020 [cited 2024 Mar 27];14(1):e0007953. Available from: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0007953
4. Rizzo C, Napoli C, Venturi G, Pupella S, Lombardini L, Calistri P, et al. West Nile virus transmission: Results from the integrated surveillance system in Italy, 2008 to 2015. Eurosurveillance [Internet]. 2016 Sep 15 [cited 2024 Mar 27];21(37):30340. Available from: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2016.21.37.30340
5. Fonseca DM, Keyghobadi N, Malcolm CA, Mehmet C, Schaffner F, Mogi M, et al. Emerging vectors in the Culex pipiens complex. Science [Internet]. 2004 Mar 5 [cited 2024 Mar 27];303(5663):1535–8. Available from: https://pubmed.ncbi.nlm.nih.gov/15001783/
6. Yee DA, Faraji A, Rochlin I. Mosquito Surveillance for West Nile Virus. Methods Mol Biol [Internet]. 2023 [cited 2024 Mar 27];2585:145–56. Available from: https://pubmed.ncbi.nlm.nih.gov/36331772/

7. Vogels CBF, Göertz GP, Pijlman GP, Koenraadt CJM. Vector competence of European mosquitoes for West Nile virus. Emerg Microbes Infect [Internet]. 2017 [cited 2024 Mar 27];6(11). Available from: https://pubmed.ncbi.nlm.nih.gov/29116220/
8. Arnold C. West Nile virus bites back. Lancet Neurol [Internet]. 2012 Dec [cited 2024 Mar 27];11(12):1023–4. Available from: https://pubmed.ncbi.nlm.nih.gov/23153403/
9. Brugueras S, Fernández-Martínez B, Martínez-de la Puente J, Figuerola J, Porro TM, Rius C, et al. Environmental drivers, climate change and emergent diseases transmitted by mosquitoes and their vectors in southern Europe: A systematic review. Environ Res [Internet]. 2020 Dec 1 [cited 2024 Mar 27];191. Available from: https://pubmed.ncbi.nlm.nih.gov/32810503/
10. Giesen C, Herrador Z, Fernandez-Martinez B, Figuerola J, Gangoso L, Vazquez A, et al. A systematic review of environmental factors related to WNV circulation in European and Mediterranean countries. One Health. 2023 Jun 1;16:100478.
11. Erazo D, Grant L, Ghisbain G, Marini G, Colón-González FJ, Wint W, et al. Contribution of climate change to the spatial expansion of West Nile virus in Europe. Nat Commun [Internet]. 2024 Dec 1 [cited 2024 Apr 4];15(1). Available from: /pmc/articles/PMC10853512/
12. Weekly updates: 2023 West Nile virus transmission season [Internet]. [cited 2024 Apr 8]. Available from: https://www.ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/disease-data-ecdc
13. Barzon L, Montarsi F, Quaranta E, Monne I, Pacenti M, Michelutti A, et al. Early start of seasonal transmission and co-circulation of West Nile virus lineage 2 and a newly introduced lineage 1 strain, northern Italy, June 2022. Eurosurveillance [Internet]. 2022 Jul 21 [cited 2024 Mar 27];27(29):2200548. Available from: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2022.27.29.2200548
14. European Centre for Disease Prevention and Control. EU case definitions [Internet]. [cited 2024 Mar 27]. Available from: https://www.ecdc.europa.eu/en/all-topics/eu-case-definitions
15. Riccò M, Peruzzi S, Balzarini F. Epidemiology of West Nile Virus Infections in Humans, Italy, 2012–2020: A Summary of Available Evidences. Trop Med Infect Dis [Internet]. 2021 [cited 2024 Jul 8];6(2). Available from: /pmc/articles/PMC8167603/
16. SCIA – Sistema nazionale per l'elaborazione e diffusione di dati climatici [Internet]. [cited 2024 May 2]. Available from: https://scia.isprambiente.it/
17. Italian National Institute of Statistics (ISTAT). ISTAT Population and households [Internet]. [cited 2024 Mar 27]. Available from: https://www.istat.it/en/population-and-households
18. Gasparrini A. The Case Time Series Design. Epidemiology [Internet]. 2021 Nov 11 [cited 2024 Mar 27];32(6):829. Available from: /pmc/articles/PMC7611753/
19. Gasparrini A. A tutorial on the case time series design for small-area analysis. BMC Med Res Methodol [Internet]. 2022 Dec 1 [cited 2024 Mar 27];22(1):1–8. Available from: https://bmcmedresmethodol.biomedcentral.com/articles/10.1186/s12874-022-01612-x
20. Armstrong BG, Gasparrini A, Tobias A. Conditional Poisson models: A flexible alternative to conditional logistic case cross-over analysis. BMC Med Res Methodol [Internet]. 2014 Nov 24 [cited 2024 Mar 27];14(1):1–6. Available from: https://bmcmedresmethodol.biomedcentral.com/articles/10.1186/1471-2288-14-122
21. Rudolph KE, Lessler J, Moloney RM, Kmush B, Cummings DAT. Incubation Periods of Mosquito-Borne Viral Infections: A Systematic Review. Am J Trop Med Hyg

[Internet]. 2014 May 7 [cited 2024 Mar 27];90(5):882–91. Available from: https://www.ajtmh.org/view/journals/tpmd/90/5/article-p882.xml

22. Gasparrini A, Armstrong B, Kenward MG. Distributed lag non-linear models. Stat Med [Internet]. 2010 Sep 20 [cited 2024 Mar 27];29(21):2224–34. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/sim.3940

23. Moirano G, Gasparrini A, Acquaotta F, Fratianni S, Merletti F, Maule M, et al. West Nile Virus infection in Northern Italy: Case-crossover study on the short-term effect of climatic parameters. Environ Res. 2018 Nov 1;167:544–9.

24. GNM package R [Internet]. [cited 2024 Oct 6]. Available from: https://cran.r-project.org/web/packages/gnm/index.html

25. DNLM Package R [Internet]. [cited 2024 Oct 6]. Available from: https://cran.r-project.org/web/packages/dlnm/index.html

26. Paz S, Malkinson D, Green MS, Tsioni G, Papa A, Danis K, et al. Permissive Summer Temperatures of the 2010 European West Nile Fever Upsurge. PLoS One. 2013 Feb 19;8(2).

27. Farooq Z, Rocklöv J, Wallin J, Abiri N, Sewe MO, Sjödin H, et al. Artificial intelligence to predict West Nile virus outbreaks with eco-climatic drivers. The Lancet Regional Health - Europe. 2022 Jun 1;17:100370.

28. Conte A, Candeloro L, Ippoliti C, Monaco F, De Massis F, Bruno R, et al. Spatio-Temporal Identification of Areas Suitable for West Nile Disease in the Mediterranean Basin and Central Europe. PLoS One [Internet]. 2015 Dec 1 [cited 2024 Mar 27];10(12):e0146024. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0146024

29. Fornasiero D, Mazzucato M, Barbujani M, Montarsi F, Capelli G, Mulatti P. Inter-annual variability of the effects of intrinsic and extrinsic drivers affecting West Nile virus vector Culex pipiens population dynamics in northeastern Italy. Parasit Vectors [Internet]. 2020 May 29 [cited 2024 Mar 27];13(1):1–12. Available from: https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04143-w

30. Kilpatrick AM, Meola MA, Moudy RM, Kramer LD. Temperature, Viral Genetics, and the Transmission of West Nile Virus by Culex pipiens Mosquitoes. PLoS Pathog [Internet]. 2008 Jun [cited 2024 Mar 27];4(6):e1000092. Available from: https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1000092

31. Wang J, Ogden NH, Zhu H. The Impact of Weather Conditions on Culex pipiens and Culex restuans (Diptera: Culicidae) Abundance: A Case Study in Peel Region. J Med Entomol [Internet]. 2011 Mar 1 [cited 2024 Mar 27];48(2):468–75. Available from: https://dx.doi.org/10.1603/ME10117

32. Paz S, Semenza JC. Environmental Drivers of West Nile Fever Epidemiology in Europe and Western Asia—A Review. International Journal of Environmental Research and Public Health 2013, Vol 10, Pages 3543-3562 [Internet]. 2013 Aug 9 [cited 2024 Apr 9];10(8):3543–62. Available from: https://www.mdpi.com/1660-4601/10/8/3543/htm

33. Carrieri M, Fariselli P, MacCagnani B, Angelini P, Calzolari M, Bellini R. Weather Factors Influencing the Population Dynamics of Culex pipiens (Diptera: Culicidae) in the Po Plain Valley, Italy (1997-2011). Environ Entomol [Internet]. 2014 Apr 1 [cited 2024 Mar 27];43(2):482–90. Available from: https://dx.doi.org/10.1603/EN13173

34. Jian Y, Silvestri S, Belluco E, Saltarin A, Chillemi G, Marani M. Environmental forcing and density-dependent controls of Culex pipiens abundance in a temperate climate (Northeastern Italy). Ecol Modell. 2014 Jan 24;272:301–10.

35. Marcantonio M, Rizzoli A, Metz M, Rosa R, Marini G, Chadwick E, et al. Identifying the Environmental Conditions Favouring West Nile Virus Outbreaks in Europe. PLoS One [Internet]. 2015 Mar 24 [cited 2024 Mar 27];10(3):e0121158. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0121158

36. Ukawuba I, Shaman J. Association of spring-summer hydrology and meteorology with human West Nile virus infection in West Texas, USA, 2002-2016. Parasit Vectors. 2018 Apr 4;11(1).

37. Paz S, Malkinson D, Green MS, Tsioni G, Papa A, Danis K, et al. Permissive Summer Temperatures of the 2010 European West Nile Fever Upsurge. PLoS One [Internet]. 2013 Feb 19 [cited 2024 Mar 27];8(2):e56398. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0056398

38. Vineis P, Romanello M, Michelozzi P, Martuzzi M. Health co-benefits of climate change action in Italy. Lancet Planet Health [Internet]. 2022 Apr 1 [cited 2024 Jul 8];6(4):e293–4. Available from: http://www.thelancet.com/article/S2542519622000614/fulltext

39. Farooq Z, Rocklöv J, Wallin J, Abiri N, Sewe MO, Sjödin H, et al. Artificial intelligence to predict West Nile virus outbreaks with eco-climatic drivers. The Lancet Regional Health - Europe. 2022 Jun 1;17:100370.

40. Manore CA, Davis J, Christofferson RC, Wesson D, Hyman JM, Mores CN. Towards an Early Warning System for Forecasting Human West Nile Virus Incidence. PLoS Curr [Internet]. 2014 [cited 2024 Mar 27];6. Available from: /pmc/articles/PMC3945055/

41. Copernicus [Internet]. [cited 2024 Mar 28]. Available from: https://climate.copernicus.eu/

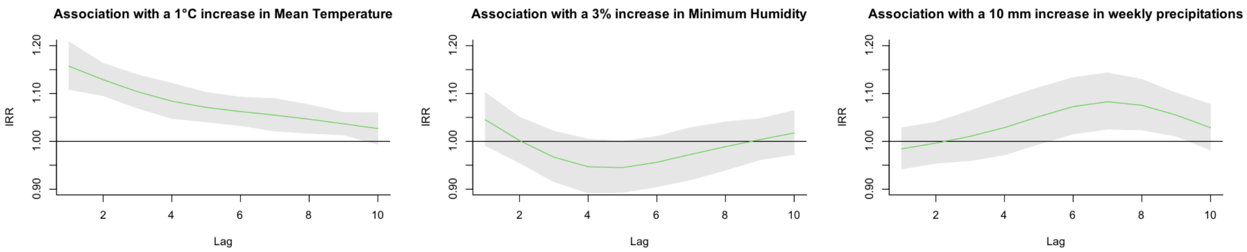
42. Gossner CM, Marrama L, Carson M, Allerberger F, Calistri P, Dilaveris D, et al. West nile virus surveillance in europe: Moving towards an integrated animal-human-vector approach. Eurosurveillance [Internet]. 2017 May 4 [cited 2024 Mar 27];22(18):30526. Available from: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2017.22.18.30526

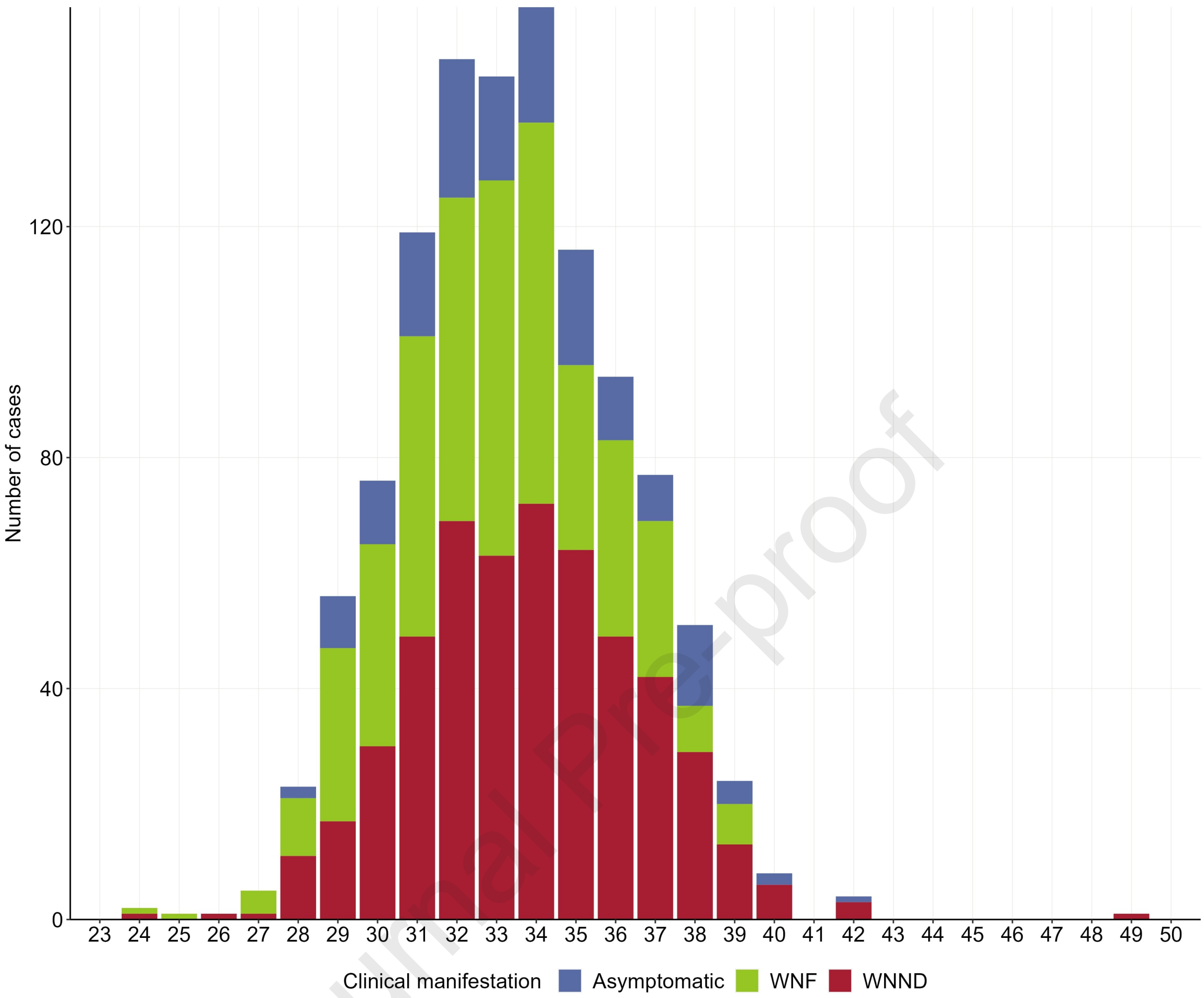
Figures legend

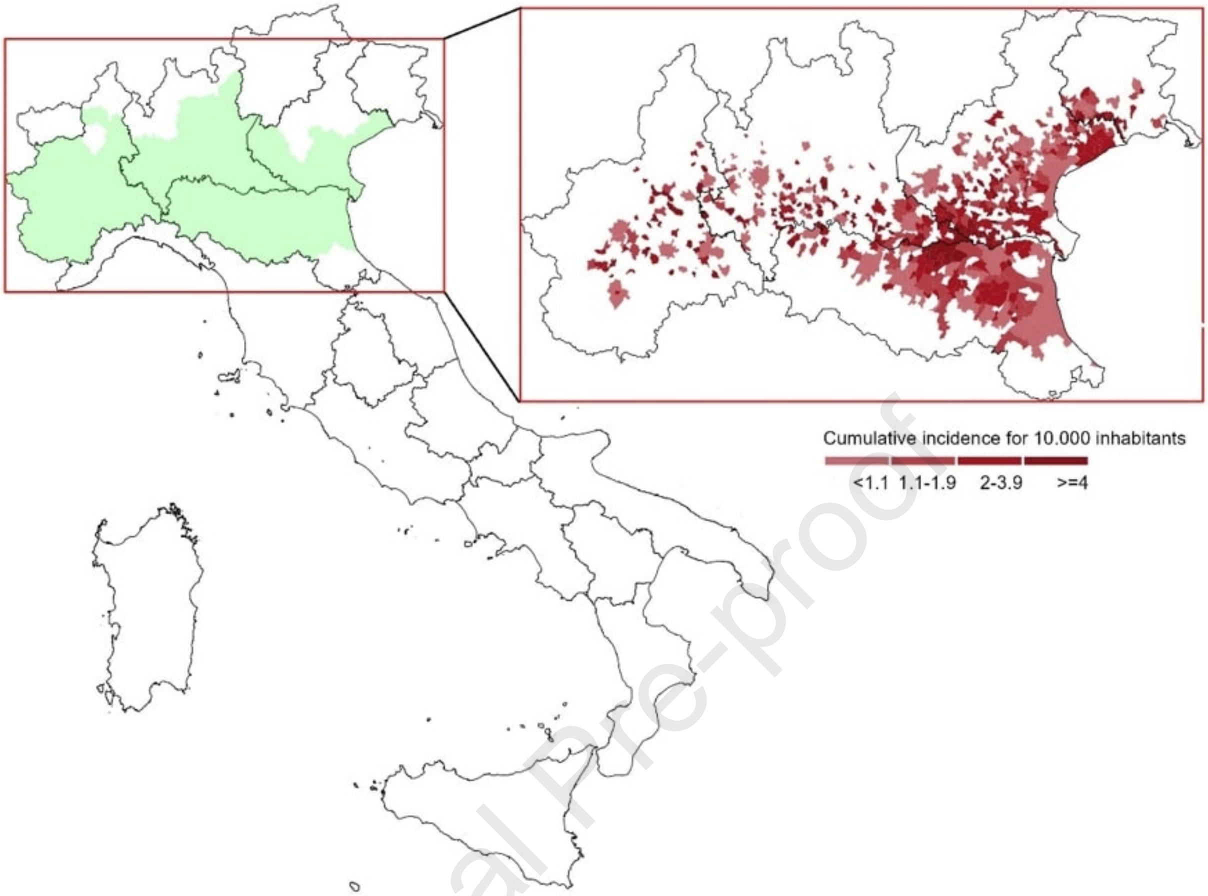
Figure 1: Autochthonous WNV cases stratified by symptoms in Northern Italy from 2012 to 2021.

Figure 2: Geographical distribution of West Nile Virus (WNV) incidence in Northern Italy. Left: Map of Italy highlighting the provinces within the Po Valley, shaded in green. Right: Detailed view of the study area, presenting the cumulative incidence rates of WNV per 10,000 inhabitants in municipalities across five Italian regions—Veneto, Lombardia, Emilia-Romagna, Piemonte, and Friuli-Venezia Giulia—from 2012 to 2021

Figure 3: Incidence Risk Ratios (IRRs) for the association of climatic variables with WNV cases across different lag weeks. The panels show the association with a 1°C increase in mean temperature (left), a 3% increase in minimum humidity (center), and a 10 mm increase in weekly precipitation (right). Each curve represents the IRR with its 95% confidence interval (shaded area), across lag weeks ranging from 0 to 10.


Association with a 1°C increase in Mean Temperature
Association with a 3% increase in Minimum Humidity
Association with a 10 mm increase in weekly precipitations
IRR
1.20
1.10
1.00
0.90
2
4
6
8
10
Lag


Number of cases
0
40
80
120
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
Clinical manifestation
Asymptomatic
WNF
WNND


Cumulative incidence for 10.000 inhabitants
<1.1
1.1-1.9
2-3.9
>=4

Highlights

- Case-time series study design can be combined with distributed lag non-linear models to examine the short-term impact of meteorological variables on vector-borne infectious diseases.
- Weeks with higher mean temperature in Northern Italy significantly heighten West Nile Virus (WNV) transmission.
- Abundant rainfalls in the previous 6 to 9 weeks increase the incidence rate of WNV cases in Northern Italy.
- High spatial resolution climatic data at the Local Administrative Unit Level can enhance WNV surveillance.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: