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West Nile Virus and Climate Change in Lombardy: a One Health Challenge

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“Humankind has not woven the web of life. We are but one thread within it. Whatever we do to the web, we do to ourselves. All things are bound together. All things connect.”
— Chief Seattle

“What is man without the beasts? If all the beasts were gone, man would die from a great loneliness of spirit. For whatever happens to the beasts, soon happens to man. All things are connected.”
— Chief Seattle

“We should draw considerable strength in the face of the challenges of climate change from the way in which the global community has addressed numerous other threats to health in the recent past (...). Responding to climate change could be the greatest global health opportunity of the 21st century.”
— The Lancet Commission on Health and Climate Change, 2015

RIASSUNTO ESECUTIVO

INTRODUZIONE E RAZIONALE

Il Virus del Nilo Occidentale (WNV) costituisce oggi una minaccia per la sanità pubblica in Lombardia, dove risulta endemico da quasi 15 anni ed è stato registrato un aumento dei casi significativo negli ultimi 5 anni. Sebbene la maggior parte dei casi siano asintomatici o pauci-sintomatici, le forme severe di malattie che si sviluppano circa nell'1% dei pazienti che contraggono l'infezione non hanno una cura e risultano letali nel 50% dei pazienti. Per affrontare questa sfida emergente, l'Italia ha implementato un sistema di sorveglianza One Health, il Piano Nazionale di prevenzione, sorveglianza e risposta alle Arbovirosi (PNA) 2020-2025, capace di rilevare la circolazione del virus a livello entomologico prima che si verifichino casi umani di infezione, che consente di ridurre il rischio di trasfusioni infette. In questo contesto endemico, il cambiamento climatico gioca un ruolo chiave: infatti le condizioni ambientali della nostra regione stanno mutando verso un clima che favorisce le zanzare-vettore delle principali arbovirosi, favorendo di conseguenza la loro presenza nel territorio. L'innalzamento delle temperature e le variazioni nei modelli di precipitazione che si stanno osservando nel territorio nel corso degli ultimi anni hanno il potenziale di influenzare la dinamica di trasmissione del WNV. Comprendere questa interazione è essenziale per lo sviluppo di strategie preventive e di controllo più efficaci. Questo studio è il primo studio a indagare l'epidemiologia di WNV in Lombardia nel periodo 2013-2022, e a metterla in relazione alle variabili climatiche.

OBIETTIVI STUDIO

- Descrivere l'epidemiologia di WNV in Lombardia tra il 2013 e il 2022
- Valutare l'impatto del cambiamento climatico (aumento delle temperature, variazioni delle precipitazioni) sull'epidemiologia entomologica e umana di WNV in Lombardia.
- Avanzare proposte concrete per favorire la ricerca in ambito WNV e implementare il sistema di sorveglianza One Health e la prevenzione delle

arbovirosi con l’aspetto climatico, oggi riconosciuto come significativo, ma non incluso nelle attività di monitoraggio.

MATERIALI E METODI

È stato adottato un approccio One Health, integrando dati epidemiologici relativi ai casi umani di WNV, alle zanzare, alle temperature massime e minime giornaliere e alle piogge cumulative giornaliere nel periodo di studio (2013-2022).

I dati umani di infezione sono stati ottenuti in collaborazione con l’Unità Organizzazione e Prevenzione (UOP) di Regione Lombardia, che ha l’accesso alle notifiche registrate negli anni sulla piattaforma nazionale Arbovirosi dell’Istituto Superiore di Sanità (ISS).

I dati entomologici sulla positività delle trappole di zanzara per WNV sono disponibili sul portale dell’Istituto Zooprofilattico Sperimentale – Lombardia ed Emilia-Romagna (IZSLER), nella sezione dedicata a WNV e Usutu virus.

I dati climatici sono stati ottenuti in collaborazione con il Dipartimento di Meteorologia dell’Università degli Studi di Milano, che ha fornito i dati di temperatura massima e minima per tutto il periodo temporale di studio, mentre quelli delle precipitazioni fino al 31 agosto 2020. Le precipitazioni dal 1° settembre 2020 al 31 dicembre 2022 sono state invece fornite dall’Agenzia Regionale per la Protezione Ambientale (ARPA) - Lombardia.

I parametri climatici sono stati analizzati per identificare correlazioni con la trasmissione del WNV. Sono state create serie storiche delle variabili climatiche nelle province di Brescia, Cremona, Lodi, Mantova e Milano, le più colpite da WNV. Sulle stesse serie storiche sono stati sovrapposti i dati relativi ai casi umani di WNV e alle positività delle trappole entomologiche, consentendo un'analisi del rapporto tra casi umani e fenomeni climatici.

RISULTATI

Tra il 2013 e il 2022 in Lombardia sono stati registrati 428 casi di infezione da WNV. Di questi, circa il 75% si sono verificati nella popolazione maschile. La popolazione più colpita da malattia che giunge all’attenzione diagnostica è compresa nella fascia

tra i 60 e i 90 anni, che risulta anche quella più colpita dai casi sintomatici di malattia, sia lievi, febbrili, sia gravi, neurologici. Una valutazione dei casi classificati con criterio clinico, classificazione introdotta negli ultimi 4 anni di studio, ha inoltre mostrato che oggi i casi severi di malattia neuroinvasiva da WNV costituiscono il 35% delle notifiche, il che lascia supporre una circolazione virale molto più alta di quella che viene diagnosticata (in linea teorica, dovrebbero rappresentare solo l'1% dei casi, la punta dell'iceberg della malattia).
Osservando la diffusione a livello della regione Lombardia, è possibile notare come le province che risultano più colpite siano quelle situate nella Pianura Padana: Mantova, Cremona, Lodi, Milano, Pavia e Brescia, i cui casi si sono concentrati soprattutto nel 2022 (33 su 46 totali registrati nel corso dei 10 anni analizzati).
La distribuzione dei casi nel corso degli anni ha subito delle variazioni importanti: se infatti fino al 2017 i casi sono stati tra i 13 e i 32 all'anno, dal 2018 si è assistito a 3 picchi, rispettivamente di 62 casi nel 2018, 78 nel 2020, e 130 nel 2022.

Le nostre osservazioni sulle serie storiche climatiche hanno poi rivelato una correlazione visibile tra i fattori climatici e l'incidenza dei casi di WNV in tutte le province selezionate dallo studio. Temperature più elevate hanno mostrato una correlazione con nuove positività delle trappole e nuovi casi umani di WNV. È interessante notare come spesso le positività delle trappole e i casi umani si siano verificati in seguito a picchi di precipitazione. Inoltre, gli anni a più alta circolazione virale (2018, 2020 e 2022) sono stati caratterizzati da primavere più calde e meno piovose, in accordo con quanto osservato in alcuni studi in letteratura. Questi primi risultati mettono in luce l'interrelazione tra il cambiamento climatico e le dinamiche di trasmissione del WNV, sottolineando l'importanza di un approccio olistico "One Health" per comprendere e contenere la malattia.

DISCUSSIONE E CONCLUSIONI

Questo studio sottolinea la necessità di adottare misure attive per affrontare la sfida One Health rappresentata dal WNV e dal cambiamento climatico in Lombardia. Solo integrando dati sulla salute umana, su quella animale e informazioni ambientali, si potrà ottenere una comprensione completa delle complesse interazioni che guidano la trasmissione del WNV. Le correlazioni climatiche individuate sottolineano la necessità di ulteriori studi che mettano in relazione statistica non solo i picchi termici, ma anche i picchi di precipitazione, con i casi di WNV. L'importanza pratica di studi di questo tipo può risultare in un'aumentata capacità di progettare modelli predittivi a lungo termine e di integrare l'aspetto climatico nei sistemi di sorveglianza esistenti, con la possibilità di prevedere il rischio per l'anno in corso anche in base all'andamento climatico delle stagioni. La ricerca interdisciplinare continua e gli sforzi collaborativi di tipo One Health sono essenziali per studiare le arbovirosi nel contesto del cambiamento climatico, che costituisce già adesso una problematica di rilievo per la salute pubblica.

EXECUTIVE SUMMARY

INTRODUCTION AND RATIONALE

West Nile Virus (WNV) poses a significant public health threat in Lombardy. The region has been grappling with this endemic infection for nearly 15 years, with a notable increase in human cases over the last five years. While most patients contract a mild or asymptomatic form of the virus, about 1% develop severe symptoms, which are incurable and ultimately fatal in 50% of cases. To tackle this emerging challenge, Italy has instituted a One Health surveillance system known as the National Plan for the Prevention, Surveillance and Response to Arboviroses (PNA) 2020-2025. This system has the ability to detect the virus's circulation at an entomological level before the occurrence of human infections. In this endemic context, climate change is crucial. Environmental conditions in our region are shifting towards a climate favorable to the main arbovirus vectors, specifically mosquitoes, resulting in their increased presence in the area. The rising temperatures and changes in precipitation patterns observed in the territory over the past few years possess the potential to affect the transmission dynamics of WNV. Understanding the interaction of WNV and climatic variables is crucial for developing effective preventive and control strategies. This study is the first of its kind in Lombardy.

STUDY OBJECTIVES

This study focuses on the epidemiology of WNV in Lombardy between 2013 and 2022. The study aims to describe the epidemiology of WNV in Lombardy and to assess how climate change, such as increased temperatures and changes in rainfall, impacts both the entomological and human epidemiology of WNV.

We made concrete proposals to advance research on WNV and establish the One Health surveillance system for the prevention of Arboviroses, adding consideration to the climactic aspect.

MATERIALS AND METHODS

A One Health approach was undertaken, incorporating epidemiological data on human WNV cases, mosquitoes, daily maximum and minimum temperatures, and daily cumulative rainfall between 2013 and 2022.

The data on human infections were collected in collaboration with the Organization and Prevention Unit (UOP) of the Lombardy Region, which had access to notifications recorded over the years on the National Arboviroses Platform of the Istituto Superiore di Sanità (ISS).

Entomological data regarding the positivity of mosquito traps for WNV can be accessed on the Istituto Zooprofilattico Sperimentale - Lombardia ed Emilia-Romagna (IZSLER) portal under the section dedicated to WNV and Usutu virus.

The climactic information was procured through a joint effort with the Department of Meteorology of the University of Milan. The Department supplied the utmost and the least temperature data for the entire course of the study and precipitation data through to 31 August 2020. ARPA-Lombardy provided the precipitation data from 1 September 2020 to 31 December 2022.

RESULTS

From 2013 to 2022, a total of 428 instances of WNV infection were registered in Lombardy. Of these, around 75% transpired in males. The population that is most susceptible to this disease and comes to medical attention is the age group between 60-90. This group also experiences the most symptomatic cases of the disease, which can range from mild, febrile to severe neurological manifestations. An assessment of cases categorized by clinical criteria, which was introduced in the last four years of the study, indicated that presently, severe instances of WNV neuroinvasive ailment account for 35% of the notifications. This implies a far more extensive viral spread than is diagnosed (in principle, they should only represent 1% of cases, i.e., the visible part of the condition).

Upon analyzing the spread of cases within Lombardy, it is evident that the provinces most impacted exist within the Po Valley; specifically, Mantua, Cremona, Lodi, Milan, Pavia, and Brescia. In the past decade, only 46 cases were reported, of which 33 were concentrated in the year 2022.

The distribution of cases has significantly varied over the years. Prior to 2017, the number of cases was between 13 and 32 per annum. However, since 2018, there have been three drastic increases, with 62 cases in 2018, 78 in 2020, and 130 in 2022.
Our analysis of climatic time series highlights a noticeable correlation between climatic factors and the incidence of WNV cases in all provinces selected for the study. Elevated temperatures are positively associated with new trap positivity and new human cases of WNV. Notably, trap positivity and human cases frequently follow periods of high precipitation. Furthermore, the years with the highest viral circulation (2018, 2020 and 2022) were characterized by warmer and less rainy springs, in agreement with what has been observed in relevant literature. These preliminary findings emphasize the interconnection between climate change and WNV transmission dynamics, accentuating the significance of a comprehensive 'One Health' approach to comprehend and control the disease.

DISCUSSION AND CONCLUSIONS

This study emphasizes the requirement for proactive measures to tackle the One Health predicament caused by WNV and climate change in Lombardy. Unless human health data, animal health data, and environmental information are integrated, a thorough comprehension of the multifaceted interactions that prompt WNV transmission cannot be achieved. The identified climate correlations emphasize the necessity for additional studies that statistically correlate not only thermal peaks but also precipitation peaks with cases of West Nile virus (WNV). Conducting these kinds of studies holds practical importance, as it can enhance the ability to design long-term predictive models and incorporate the climatic aspect into existing surveillance systems. This, in turn, may enable us to predict the risk for the current year based on seasonal climatic trends. Continued interdisciplinary research and collaborative One Health initiatives are indispensable for examining arboviruses in relation to climate change, which is a substantial public health concern.

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Background

When we talk about "climate change", we tend to think of it as something that will happen in the future, but the truth is that it is happening right now, in our immediate environment. However, its impact on human health has been largely overlooked (1,2). In fact, the climate crisis poses a significant threat to public health, with vector-borne infectious diseases, including Arboviroses and tick-borne diseases, serving as primary examples of this phenomenon (3).

One instance of this can be seen with the West Nile Virus (WNV) infection in Lombardy, which is still regarded as an emerging disease despite its existence as an endemic virus for 15 years in the Po Valley (4). Over the past five years, there has been a surge in the number of reported cases, eliciting attention not only from experts but also from the general public (4,5).
Concern regarding this illness has been slow to increase due to its asymptomatic or mildly symptomatic nature in 99% of cases. However, 1% of those who become infected with the disease can develop more severe and deadly forms with neuroinvasive effects, which presently have no available treatment or vaccine for prevention (6).

The best way to control the spread of WNV is through epidemiological surveillance to identify the presence of vectors and of infected ones in the area as early as possible (7). This is made possible by implementing the *Piano Nazionale di Prevenzione, Sorveglianza e Risposta alle Arbovirosi (PNA) 2020-2025*, which includes *One Health* surveillance, as it recognizes that arboviruses are a health concern that affects not just humans, but also animals and the environment in a highly interconnected way (8). However, an area that requires attention in this plan pertains to environmental monitoring. Although acknowledging the link between weather conditions and the dissemination of WNV, the current *One Health* approach fails to integrate climate surveillance.

This thesis undertakes a comprehensive investigation of the epidemiology of WNV in Lombardy from 2013 to 2022. It includes an analysis of the potential contribution of specific climatic drivers such as temperature and precipitations to the spread of West Nile Virus in the region during this period. The study is unique to Lombardy and yields valuable insights into the epidemiology of WNV. The approach used is fully aligned with the One Health approach promoting integrated action encompassing human health, animal health and entomological considerations, and environmental parameters. It is also aligned with the approach proposed within the framework of the United Nations (UN) Sustainable Development Goals (SDGs), where complex challenges need to be faced through multisectoral and cross-disciplinary action that builds on the consideration that effective interventions relevant to various SDGs are integrated and indivisible (9).

Recognizing and describing the validity of the link between climate and WNV has more than a retrospective value: it implies the need to act with a truly comprehensive One Health approach, integrating climate aspects with epidemiological surveillance, opening up the possibility of building more robust predictive models that also help to identify areas of future virus circulation, thus preparing the health system for a possible increase in cases.

The thesis comprises five parts.
The first chapter, the introduction, will provide detailed descriptions of the WNV infection, its symptoms, and epidemiology in Italy and Lombardy. Additionally, it will examine Italy's WNV Viral Circulation Surveillance System which exemplifies the One Health approach. The final section explores the concept of a link between climate and WNV, based on the evidence in the literature with reference to climate change.

In chapter 2, the study's design will be outlined, including both an epidemiological-descriptive and an experimental component. This study is a pioneering effort about WNV epidemiology in Lombardy. The data employed and associated collection procedures will be expounded in detail. The methodology utilized to analyze the obtained outcomes will also be deliberated. This section delivers a firm methodological foundation for scholars to reproduce the experiment and substantiate the accuracy of the outcomes.

Resulting from the research, the third chapter illustrates and evaluates the findings of the study. The descriptions of the observations are thoroughly thorough, aided by tables and graphics that lucidly depict the collected data. This chapter presents a comprehensive record of the study's discoveries, giving an unambiguous summary of the relationships and trends discerned from the collected data.

In the fourth chapter, the results are interpreted with respect to the research questions and initial hypotheses. The significance and implications of the findings will be examined, and where possible, compared with prior research conducted in regions neighboring Lombardy. The study's limitations and potential areas for future research will be critically examined in this chapter. It is essential for comprehending the significance of the results obtained and situating the study's distinctive contribution within the framework of extant scientific knowledge.

The concluding chapter outlines the key findings of the study while emphasizing the import of the results acquired. The study's practical and theoretical implications are summarized, outlining the main lessons learnt during the investigation. Suggestions for future research are offered, based on the approaches and findings presented and designed to expand upon the topics discussed.

In the following pages, we will investigate the complex interrelationships between climate change and the West Nile virus in Lombardy. With a multidisciplinary approach, our goal is to shed light on this rapidly developing *'One Health'* issue driven by a profound sense of responsibility: we are not just dissecting scientific complexities; we are paving the way for transformative change. It is important to tackle this challenge with resoluteness and decisiveness, recognizing that the climate crisis is already a health crisis (10). We are committed to shaping a resilient tomorrow, where the well-being of humanity, the integrity of our ecosystems, and the harmony of our shared existence thrive against the backdrop of global challenges.

Chapter I: Introduction

West Nile Virus (WNV) is a single-stranded RNA, vector-borne Flavivirus that belongs to the family Flaviviridae, as do Hepaciviruses and Pestiviruses. The genus Flavivirus comprises about seventy single-stranded RNA viruses that are transmitted by tick or mosquito bites, some of them responsible for human diseases such as Dengue and Yellow Fever (11). WNV also causes a form of human disease: although infection is asymptomatic in 80-90% of cases, some patients may develop febrile symptoms (West Nile Fever, WNF) or severe neurological symptoms (West Nile Neuroinvasive Disease, WNND), which can lead to death (6).

WNV is now considered to be endemic in the Po Valley area, from Friuli Venezia Giulia to Piedmont via Lombardy (4). This seems to be related, on the one hand, to the environmental characteristics of the area (12) and, on the other hand, to climatic anomalies linked to climate change (13), which have favored the development of a habitat suitable for viral amplification and vector-human interaction. Despite this, awareness of WNV among health professionals and the public is still extremely low, which affects knowledge of the true epidemiology of WNV infection in our territory (5,14).

Pathogen description

WNV is an enveloped virus with an icosahedral shape and a diameter of about 50 nm. The RNA genome is approximately 11 kb in size and encodes three structural and seven non-structural proteins (6,11).

The structural proteins are the **WNV capsid**, which is responsible for the construction of the nucleocapsid; the **envelope protein**; and the **pre-membrane/membrane protein**, which, after cleavage, allows the virus to enter the host cell by activating membrane fusion.

The non-structural proteins are **NS1**, which mediates viral replication and assembly, inhibits viral immune responses in the host, and promotes hyperpermeability of human brain endothelial cells; **NS2A**, which is involved in the duplication of the WNV genome; **NS2B** and **NS3**, which together are involved in viral replication and cleavage of genomic proteins, and also contribute to host cell apoptosis; **NS4A** and **NS4B**, which promote host cell membrane rearrangement and contribute to the inhibition of the host immune response; **NS5**, which has polymerase and methyltransferase activities and, in the absence of a proofreading mechanism, facilitates genomic sequence changes that can lead to viral evolution (6).

At least nine viral lineages have been described in the literature, but only lineages 1 (WNV-1) and 2 (WNV-2) have been associated with human epidemic outbreaks (13).

Lifecycle

WNV is maintained in nature by a mosquito-bird-mosquito cycle, the dynamics of which are currently not fully understood. Mosquitoes are virus vectors, while birds are primary amplifying reservoir (15).

The development of virus circulation areas is intricately linked to the migratory routes of birds: indeed, infected migratory birds introduce the virus into new areas where high densities of permissive mosquitoes and susceptible local birds allow the virus to amplify and spread locally (16). This mechanism is confirmed by phylogeography studies: an example is the spread of WNV-2 in Europe. By following the phylogenetics of this lineage, it is possible to observe that there is a high degree of genetic similarity between viruses circulating in areas that are geographically distant from each other. This is explained by the fact that WNV-2 arrived in Hungary from Africa via a single event and then spread from there to different countries (Greece and Austria, and from there to Italy and the Czech Republic) via new reservoir species of migratory birds (17).

Not all avian species are equally capable of developing viremia and becoming WNV reservoirs. Based on available literature, there are marked differences between species involved worldwide, partly related to their presence and susceptibility in different

areas (16). It is interesting to see how what has been reported in the US differs from what has been observed in Europe.
In the USA, American Robins (*Turdus migratorius*), Northern Cardinal (*Cardinalis cardinalis*) House Sparrows (*Passer domesticus*) and House Finch (*Haemorhous mexicanus*) have been identified as the most common WNV reservoirs. High mortality in wild birds was observed during epidemiological peaks of WNV infection in humans (15). The association between mortality and WNV was confirmed by necropsies (18). The literature also shows that in the USA, *Culex pipiens* mosquitoes – which constitute WNV main vector worldwide – prefer robins as a blood meal. In addition, robins develop WN viremia for longer than other avian species (15).

In Europe, however, no peaks in avian mortality corresponding to human cases have been recorded so far, which means that the viremia developed by reservoir species does normally not cause them to die (19). In Italy, WNV-positive specimens have been found in many different wild species, including little owls (*Athene Noctua*), king-necked pheasants (*Phasianus colchicus*), magpies (*Pica pica*), sparrows (*Passeridae*), blackbirds (*Turdus merula*), collared doves (*Streptopelia decaocto*), grey crows (*Corvus corone cornix*) and jaybirds (*Garrulus glandarius*), suggesting that the virus is circulating in these species(15,19,20).

Similarly, not all mosquitoes carry the virus. Observations show the involvement of ornithophilic mosquitoes, mainly belonging to the species *Culex pipiens* (21). Laboratory studies have shown that other species such as *Aedes aegyptus, Aedes japonicus* and *Aedes koreicus* may also be able to transmit the virus but no transmission in nature has been recorded yet (22–24).

The life cycle of *Culex pipiens* lasts from two to four weeks and is influenced by several environmental variables (which will be discussed in detail later in a specific section), to the point that it may last as little as one week during the hot summer period (25). An egg develops in an adult mosquito in about 7-10 days (26). The adult female mosquito lays up to 300 eggs, one at a time, on the surface of fresh or stagnant water (e.g., animal troughs, ponds, unmaintained swimming pools, water-filled containers, tubs, puddles, streams, and marshes). The eggs form a raft that floats on the water (26). The larvae emerge from the eggs and live in the water, feeding on the organic elements available. After a brief time, they metamorphose into a pupa. The adult mosquito emerges from the pupa within 2 to 3 days. The estimated flight radius of *Culex pipiens* varies from 200 m to 2 km (27).

Both male and female mosquitoes require sugar as a nutrient, which they obtain from flowers and fruits. Females need a protein-rich blood meal for spawning. *Culex pipiens* are opportunistic feeders, as host availability influences their feeding behavior (16). After the blood meal, the mosquitoes search for a suitable water source for their eggs. Several days may elapse between the blood meal and the laying of the eggs (26).
Culex mosquitoes overwinter as fertilized adult females in sheltered places. The reduction in daylight hours triggers the onset of diapause (28).
As mentioned above, *Culex pipiens* are ornithophilic mosquitoes with opportunistic feeding behavior. During the spring season they prefer the blood of birds: in fact, the availability of avian species is greater because of the entry of migratory species and the subsequent nesting, which makes birds more sedentary and therefore more vulnerable to mosquitoes. It is during this period that the enzootic cycle between birds and mosquitoes takes place, with amplification of WNV infection: mosquitoes become infected through the blood meal and in turn infect non-immune birds, thus amplifying the virus reservoir. The greater the interaction between mosquitoes and birds, the more the virus is amplified and the higher the circulation of WNV (15,16).

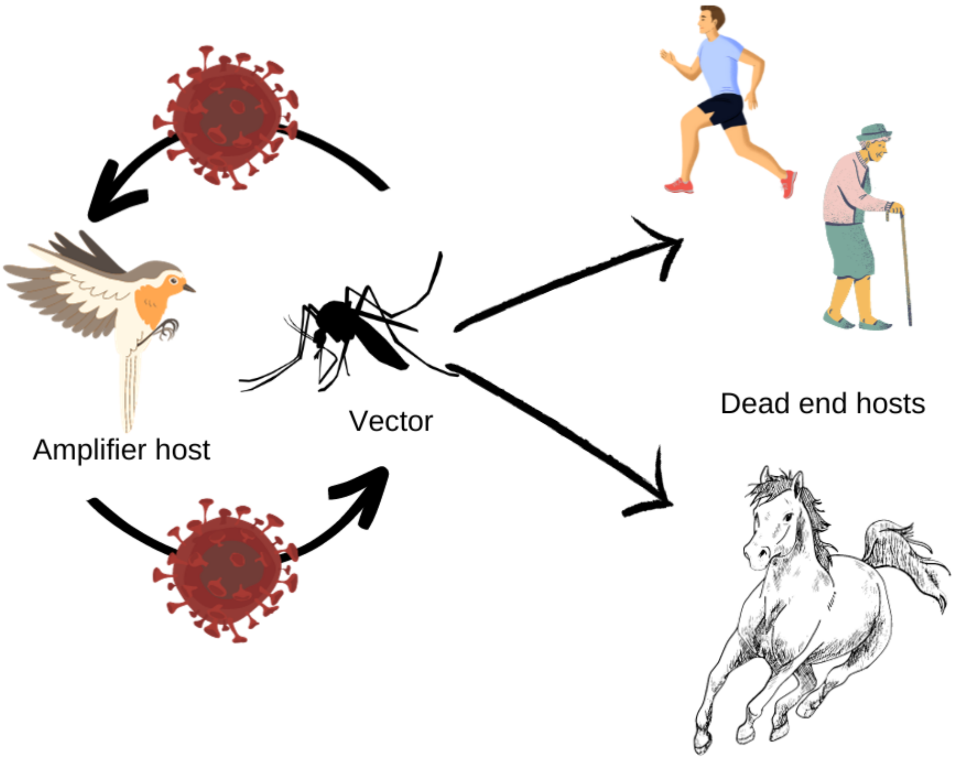

Figure 1, WNV transmission cycle

Studies of the blood content of mosquitoes show that *Culex* mosquitoes switch from avian to mammalian blood meals in late spring/early summer. This period coincides with the end of the nesting season, which increases the mobility of birds, making them less vulnerable to mosquitoes. In addition, bird migration begins again at the end of the summer. These conditions reduce the availability of birds and, as a result, mosquitoes begin to feed on mammalian blood meals. When infected with WNV, *Culex* mosquitoes transmit the infection to mammals such as humans and horses (15). Mammals are considered dead-end hosts because they do not develop viremia capable of infecting susceptible mosquitoes (16).

As mentioned above, phylogenetic studies have shown that WNV can be considered endemic in the Lombardy region, particularly in the provinces of Mantua, Cremona, Pavia, Brescia, Milano and Lodia (4). This means that the virus remains dormant in the reservoirs during the winter and re-emerges in the spring-summer with the mechanisms described at the beginning of this paragraph. It is known that the virus can remain latent in diapausing *Culex* mosquitoes, and at the same time WNV-positive birds have been found in winter, suggesting that some birds may be carriers for a prolonged period (29). Further studies are needed to clarify this process.

Clinical manifestations

Human infection with WNV is primarily asymptomatic (more than 80% of infected individuals), while up to 20% develop West Nile disease (WND). WND has an incubation period of 2-14 days and presents mainly as West Nile fever (WNF) with flu-like symptoms including fever, headache, asthenia, myalgia, nausea, vomiting and rash (6). Cases of WNF are under-diagnosed due to non-specific symptoms, which also may not require clinical attention (4).

Less than 1% of infected patients develop West Nile neuroinvasive disease (WNND), a condition characterized by WNV invasion of the central nervous system (6). WNND includes meningitis, encephalitis, poliomyelitis, and other forms of flaccid paralysis (30). WNND occurs predominantly in the elderly, chronically ill and immunosuppressed. According to a recent study by *Gervais* and colleagues, susceptibility to the development of WNND could be linked to the presence of anti-INF autoantibodies (31). WNND is associated with patient death in 10% of cases and post-illness morbidity in approximately 50% of survivors (4). WNND cases are the best available index to estimate the circulation of the virus in the area, as they require hospitalization and therefore appropriate diagnostic procedures (25).

WNV is an arbovirus; therefore, its transmission is linked to the presence of vector mosquitoes. This explains the seasonality of the infection, with human cases recorded between mid-June and mid-October and peaking in August in Italy (4,32).

A study about Italian cases occurring between 2012 and 2020 showed a predominance of WNV infections in the male population (2:1), and 75% of WNND cases are also male. It is assumed that the male population is more affected because it is the population more exposed to outdoor activities such as agriculture, forestry, and construction, but there is no evidence that this is the cause. Similar incidence data are found worldwide (4).

Diagnosis

The clinical criteria for the diagnosis of WNV are the presence of fever or at least one of the following clinical manifestations: encephalitis, meningitis, Guillain-Barré-like polyradiculitis-neuritis, acute flaccid paralysis (8).

EU laboratory diagnostic criteria to confirm cases of WNV infection are isolation of WNV or detection of WNV nucleic acid in blood or cerebro-spinal fluid (CSF); WNV-specific antibody response in CSF; high titer of WNV IgM and detection of WNV IgG and confirmation by neutralization (13).
Italy adopts EU laboratory diagnostic criteria and adds the detection of WNV nucleic acid in urine samples as a confirmatory test for infection (13).

A probable case is defined as a patient who meets the clinical and laboratory criterion for a probable case, which is the presence of a specific IgM antibody response to WNV in serum (8).

Diagnostic tests are also performed on all blood samples from donors who came from WNV endemic areas (8), because of the risk of infection through blood transfusion (33).

Treatment and prevention

There is no definitive or specific treatment for WND, so clinical management is supportive according to symptoms (pain control for headache, antiemetic therapy, rehydration). Patients should be monitored for the risk of complications such as increased intracranial pressure, seizures, and neuromuscular respiratory failure (6,34).
For the past 25 years, scientific research has attempted to develop vaccines against WNV. To date, there are no human vaccines against WNV, although several are in preclinical and clinical trials. There are practical barriers to conducting phase III trials, related to the difficulty of selecting suitable target populations due to the short circulating season of WNV. There are four effective equine anti-WNV vaccines requiring two primary doses and annual boosters (35).

Therefore, in the absence of any apparent effective therapeutic treatments or human vaccines, it is currently essential to act at the environmental level by reducing mosquito breeding habitats to reduce the virus vectors and thus the risk of human infection (4). People living in high-risk areas should use anti-mosquito repellents to avoid vector bites. However, studies show that people are not aware of the risk of contracting WNV through mosquito bites. As a result, they tend to underestimate the importance of personal prevention (14).

Descriptive Epidemiology

WNV was first isolated in 1937 in a woman with feverish symptoms, originally from the West Nile province of Uganda: hence the name of the pathogen, West Nile virus. The first cases of WNND were reported in Israel (1957) and France (1962). Only sporadic cases were reported in Europe until 1996, when WNV infection caused an epidemic outbreak in Romania (800 suspected cases), and then in Russia in 1999 (36). In Italy, the first autochthonous cases of WNV infection were reported in 14 horses in Tuscany, in 1998, but no human case was reported until 2008 (37).
In the summer of 2008, a WNV lineage 1 strain caused the first human epidemic outbreak ever recorded in Italy, affecting 8 provinces between Emilia Romagna, Veneto, and Lombardy (7,37). From 2008 to 2011, 43 human cases of WNND were reported in Italy between Veneto, Emilia Romagna, Lombardy, Friuli Venezia Giulia, and Sardinia (38).

Since 2012, with the introduction of a new WNV lineage 2 strain from Eastern Europe (39), there has been a progressive increase in both cases and affected regions. In the period 2012-2020, positive WNV cases were found in 41 different Italian provinces. Most cases were reported in the Po Valley, but virus circulation was also detected in Tuscany, Marche, Basilicata, Apulia, Sicily, and Sardinia (4).

As mentioned above, the Po Valley has been identified as an area of high virus circulation: a geographical shift of infection from east to west has been documented over the years. This trend has been observed also in Lombardy: until 2013, the only province considered at risk was Mantua, the most easterly. Gradually, WNV circulation spread to neighboring provinces further west, with cases recorded in Cremona, Brescia, Lodi, Pavia, and Milan (37).

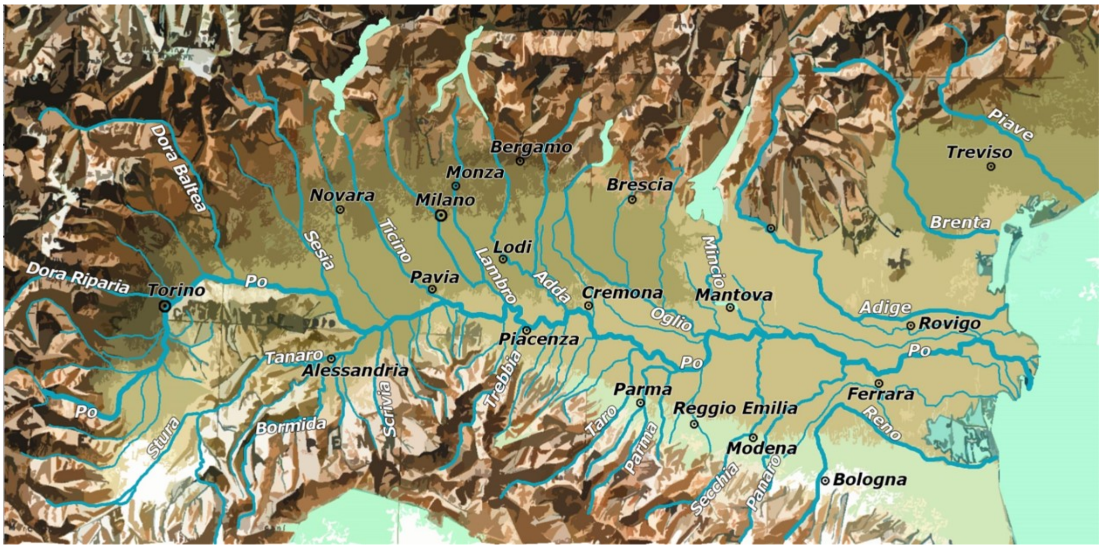

Figure 2, Po River Valley

The graph below shows the incidence of WNND cases in Italy from 2012 to 2022. As already mentioned, WNND are the most reliable index to assess the distribution of WNV in a territory, as they increase in direct proportion to the virus circulation (4). Considering that before 2012 there were 43 cases of WNND over 4 years, 2012 and 2013 were two years of high virus circulation compared to the previous period (with 28 and 45 cases/year, respectively). Subsequently the years with the highest WNV circulation were 2018 and 2022, with 244 and 295 cases of WNND respectively (32).

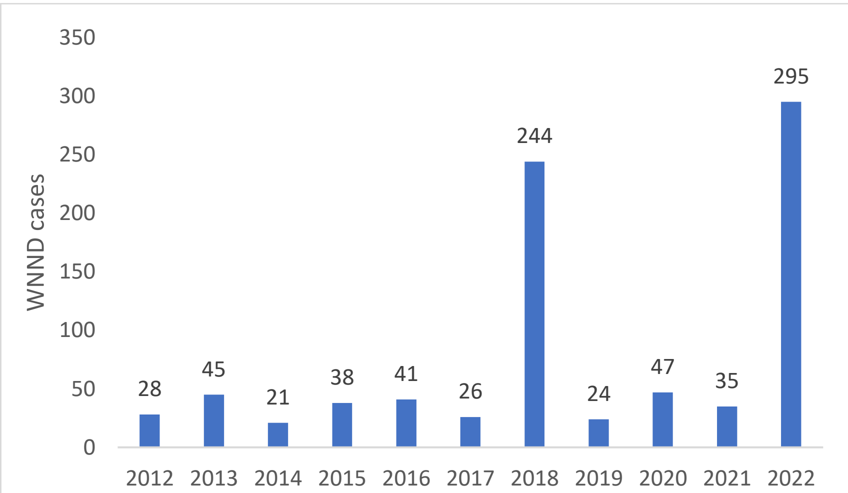

Figure 3: WNND cases in Italy, 2012 – 2022. Based on IZS and ISS data (32)

Statistically we know that cases of WNND represent approximately 1% of all cases of infection. This means that in 2018 and 2022, based on the diagnosed cases of WNND, it is possible to estimate a total of 25,000 to 30,000 cases of infection per year, of which 80% are asymptomatic and 20% have WNF. Additionally, the WNV infection notification bulletins (which also include donors with a positive nucleic acid amplification testing NAT and WNF diagnosis) report 610 cases in 2018 and 588 in 2022. In 2018, cases of WNND accounted for 40% of diagnoses. By 2022, this figure had increased to 48.5% of all reported cases, significantly higher than the 1% expected based on epidemiological trends. This means that the cases that come to the attention of health services are very underestimated in relation to the scale of the phenomenon. We can estimate that the notified cases represent approximately 2% of the total number of infected individuals. (4,32).

As partially discussed in the previous paragraphs, Italy has experienced the introduction of different viral lineages. The first viral strain to appear was a WNV lineage 1, originating from Romania, which was the predominant circulating form until 2012 (37). From 2011, the introduction of lineage 2 was observed, imported from Austria, which became the predominant form from 2012 onwards, completely replacing lineage 1 (17). From 2021, the introduction of a new viral strain was noted, again belonging to lineage 1, which became the predominant strain in 2022 in some provinces, including Padua, where the highest number of cases in Italy was recorded (40). It has been hypothesized that the new WNV lineage 1 strain appears to be associated with an increased risk of WNND due to genomic mutations (40).

To conclude the epidemiological considerations, it is important to note that WNV is now considered the most widespread arbovirus in the world, with cases recorded in all continents except Antarctica (4). In Europe, an increasing infection trend has been observed over the last decade in central, eastern, and southern areas; no infection trend has been observed in northern and western areas. These tendencies appear to be related to the increase in temperature associated with climate change, which is making areas that were not previously suitable for virus circulation, such as Germany, suitable for WNV spread (41). To date, Italy is the European country most affected by WNV, and the Po Valley is the most vulnerable region for both ecological and geographical reasons: it is the most densely populated area in Italy and at the same time a well irrigated area with natural ponds, conditions that guarantee a suitable habitat for mosquitoes and birds and consequently for the circulation of WNV between reservoirs and humans (4,32).

Control of WNV circulation

Following the first human WNV cases in Europe between the late 1990's and early 2000's, the Italian Ministry of Health established an initial surveillance system to monitor the introduction and spread of West Nile virus in Italy. Similar surveillance systems have been implemented in all European countries at risk (41).

Based on environmental characteristics, the Italian surveillance system identified 15 risk wetland areas (42). From 2002 to 2007, only sporadic WNV infections in birds and horses were notified, but no human case was reported. In 2010, after first human outbreaks in Italy, the surveillance plan was implemented with a stronger focus on the areas previously identified as virus circulation, concentrating on target species (magpies, crows, etc.), rural poultry farms, insects, equids (throughout Italy, including non-endemic areas), wild bird mortality and migratory species (4,38,43).

At the same time, the areas with the highest WNV circulation established their autonomous regional surveillance systems, which required the collaboration of entomologists, veterinarians, rangers, and public health officers (42). WNV surveillance is an example of "One Health approach", a *global health model* based on the recognition that human, animal, and ecosystem health are inextricably linked and require integrated, multidisciplinary and cross-sectoral interventions (44). In the case of a virus such as WNV, for which there is no preventive cure or vaccine, the only way to control the spread of the pathogen and reduce its impact on human health is to act preventively by identifying and monitoring the presence of the virus in areas at risk. Once WNV circulation in mosquitoes is confirmed, action can be taken by trying to prevent human infection through vector disinfestation protocols and donor screening and transplants. In addition, as mentioned earlier in this chapter, the symptoms of WNF and WNND are non-specific and sometimes misdiagnosed: the surveillance system, by identifying WNV circulation, allows health care providers to pay greater attention to diagnostic suspicion of compatible cases (4).

Lombardy and Emilia Romagna have together set up an integrated surveillance system that involves the Istituto Zooprofilattico Sperimentale of Lombardy and Emilia Romagna (IZSLER); the Regional Health and Welfare Unit of Lombardy Region; the Regional Health Unit of Emilia Romagna; the Veterinary Units of Lombardy and Emilia-Romagna Region; the Provincial Veterinary Departments; and the Provincial Police Corps (42).

WNV surveillance in Lombardy has been carried out since 2014 (until 2013, only Mantua was considered at risk) and includes entomological surveillance (search for WNV RNA in captured mosquitoes from May to October), active surveillance in wild birds (search for WNV RNA in captured host specimens) and passive surveillance (on dead specimens), equine surveillance in horses with neurological symptoms and screening of blood donors (from June to October) (7). The use of the veterinary surveillance system has shown that positivity in mosquitoes, birds and horses is always detected earlier than in humans. This finding has therefore made it possible to modify the human prevention strategies: whereas the initial surveillance system envisaged screening with the NAT test on donors from the beginning of June for the entire viral circulation season, the finding of a sequentiality between animal and human cases makes it possible to start NAT testing on donors as soon as animal positivity is detected, thus saving the costs of larger-scale testing (7).

The Lombardy surveillance system has shown to have both preventive and economic benefits in the 2014-2018 period. It allowed the region to avoid 40% of the WNV surveillance plan costs related to NAT testing. Surveillance has also made it possible to delineate areas at risk and to detect the circulation of the virus about a month before the first human cases occur. Finally, surveillance has increased knowledge of the epidemiology and risk factors for WNV transmission (7).

In 2020, the Italian Ministry of Health updated the national WNV surveillance system to include other emerging vector-borne infectious diseases, creating the National Integrated Plan for Prevention, Surveillance and Response to Arboviruses 2020-2025 (8).
The PNA divides the Italian territory into three areas: high, low, and minimal risk, based on the infectious risk and the epidemiological-environmental conditions. The PNA, like the surveillance system in Lombardy and the other regions of the Po Valley, provides for surveillance of resident birds of the target species (magpies, hooded crows, jays), surveillance of wild birds found dead, entomological surveillance, clinical surveillance of equines and human cases in all areas. Immediate notification of all suspected cases of neurological symptoms in equids, all wild bird deaths, and all cases of neuroinvasive disease and/or recent infection in humans is mandatory throughout the country (8).

The Impact of Climate and Environmental Factors on the Epidemiology of West Nile Virus.

WNV infection is an arbovirus and a zoonotic disease, whose epidemiology is intricately linked to the study of its vectors, reservoirs, and the environment in which these organisms interact. Furthermore, as the virus has been observed in Lombardy only for the past 15 years, it is critical to investigate the climatic features that facilitated its establishment in the region to determine which other areas may be at risk (4). Moreover, in the current context of climate change, it is important to study the phenomenon with a view to the future. It is essential to evaluate the impact of recent climatic anomalies on the epidemiology of West Nile virus to develop predictive models, which will enable us to forecast future scenarios and, therefore, adequately prepare the health system (45,46).
It is therefore logical that the literature on West Nile virus prevention over the last ten years has emphasized environmental and climatic factors that impact the virus both directly and indirectly, influencing the presence and availability of vectors and reservoirs (47,48).

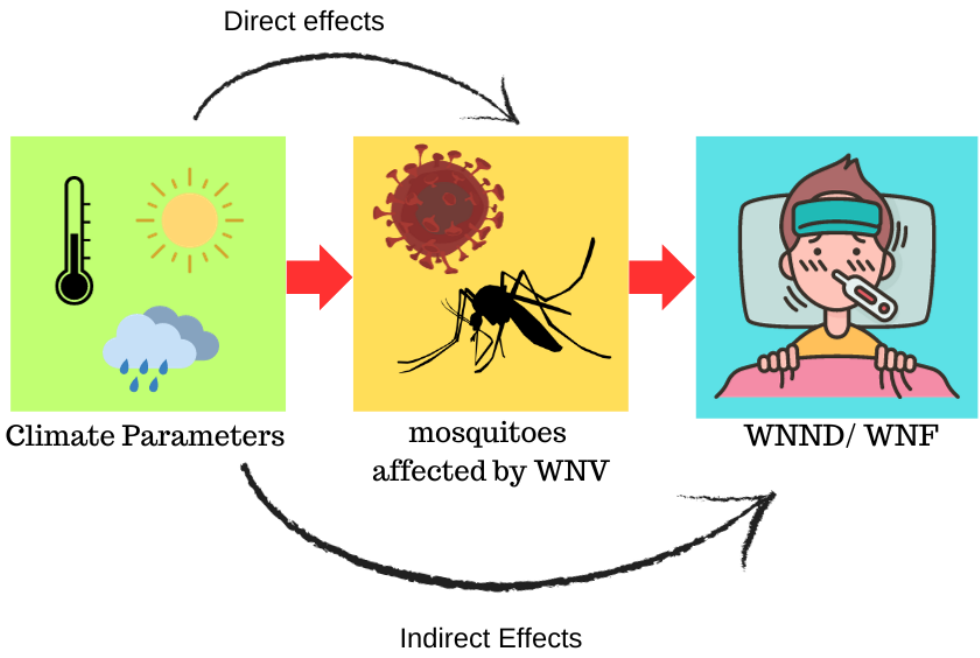

Figure 4, Direct and Indirect Effects of Climate Parameters on WNV epidemiology

However, before conducting any environmental or climatic evaluation, it is necessary to first assess the dynamics of vectors, reservoirs, and dead-end hosts. An area could become WNV endemic only if it has been subject (or is subject) to the settlement of migratory birds during the spring/summer period. Birds play a significant role in the emergence of West Nile Virus in new areas and introducing new virus strains, as previously discussed (16). Imported human cases are rare, and as mentioned earlier, humans are dead-end hosts. Cases of inter-human infection are associated with transfusions and transplants (6). Merely being located along migratory routes is not enough for human cases of WNV to develop. Other factors, such as **the presence and abundance of ornithophilic mosquitoes** (*Culex* or other vectors), **susceptible bird populations** (that have not yet developed immunity for WNV and can become endemic reservoir), and **non-immune human dead-end hosts** are also necessary (15,47,49).

Climatic influence

Parameters having direct effects: Temperature.

The climatic variable with the most evident direct impact on vector-borne diseases is **temperature**, as it has been shown to influence the cycle of pathogens in vectors, reservoirs, and hosts. Considering WNV, studies report that temperature affects the mosquito vector, showing a positive correlation between temperature increase and vectorial capacity (47).

Direct effects of temperature have been quantified as:

- **Infection rate**. Infection rate is defined as the probability of mosquitoes becoming infected after consuming an infected blood meal. According to *Vogels* and colleagues (2017) temperature increase led to a decrease in the mosquito's antiviral response due to a reduction in RNAase activity and midgut barrier efficiency (50).
- **Extrinsic Incubation Period (EIP)**. The Extrinsic Incubation Period is the duration between the time a mosquito is infected with a virus and when it becomes capable of transmitting the virus to a host. During the Extrinsic Incubation Period, the virus replicates within the mosquito's body, potentially resulting in the virus being transmitted to other hosts through bites. Studies have shown that the Extrinsic Incubation Period (EIP) decreases with an increase in temperature (Reisen et al., 2006). During the summer of 2018, the average temperatures in Emilia Romagna, Italy were 20.9°C in June, and 23.8°C in July and August. A study conducted in the region found that the extrinsic incubation period for the West Nile Virus was **one week** (25,51).
- **WNV replication rate**. Mosquitoes' viremia levels are affected by temperature. As per *Reisen* and colleagues (2006), an increase in temperature results in an increase in the viremia developed by the vectors (52).
- **WNV transmission rate**. It refers to the ability of mosquitoes to infect hosts via bites and is measured by monitoring the concentration of WNV virus in the mosquito's salivary glands. *Vogels* and colleagues (2017) conducted a laboratory experiment in which *Culex* mosquitoes were exposed to WNV after incubation at different temperatures: mosquitoes exposed orally to WNV did not develop a transmission rate below 18°C, whereas they did at 23°C and 28°C (50).

Parameters having indirect effects: Temperature, Precipitation, Relative Humidity, drought indices and seasonality.

The epidemiology of WNV, like other vector-borne diseases, relies on the presence of competent vectors in the area. Environmental and climatic conditions indirectly impact the incidence and prevalence of WNV by fostering Culex population (28,47). The vector population is influenced by various environmental factors, which will be discussed in the following paragraphs.

- **Temperature**.

Temperature is directly correlated with the **presence**, **abundance**, and **population growth** of vectors (25). According to all studies, temperature is reported as the most important environmental variable that influences the epidemiology of WNV. It is also considered a key factor in predictive studies (47).

Temperature affects all stages of the *Culex pipiens'* **life cycle**. *Culex* survival and dispersal are optimum in the temperature range of 25°C to 35°C, with the highest survival rate at 25°C. Larval embryonic development is impossible below a temperature of 7°C (47).

Rising temperatures affect the **feeding behavior** and **enhance the biting rate of mosquitoes** (52). A direct relationship between temperature and **flight activity** has been documented as well (21,28).

Temperature effects are **season dependent**. Several studies acknowledge that temperature plays a predominant role in late spring, between late April to mid-June (39,47,53). If temperature rises above the seasonal average in late spring, it leads to an earlier onset of the mosquito season, an increase in population density, and a prolongation of the mosquito season. Conversely, a late temperature rise diminishes the mosquito population density (53).
This aspect was further explored in a study conducted in Piedmont by *Marini* and colleagues (2016) ((53). The study examined the influence of temperature fluctuations

within -2.5/+2.5°C range, based on the spring seasonal average of 18.5-21.5°C. With increasing temperatures, the length of the mosquitoes' season becomes longer, and the peak mosquito density occurs earlier. The maximum abundance of collected mosquitoes reduces by 20-30% with a temperature increase of +2.5°C. This observation can be attributed to the balance between mosquito population growth and increased adult mortality, which means that <u>an increase in temperature does not always result in an increase in mosquito abundance</u>. This explains why there is no direct proportional relationship between the abundance of mosquitoes and their rate of infection with WNV (28).

Conversely, lowering the temperature is expected to considerably reduce the number of mosquitoes collected. A 2.5°C temperature decrease leads to a 40% population decrease, while a decrease of 1.5°C below the average results in a 20% population reduction (53).

The effect of temperature on West Nile virus (WNV) infections in humans is observed with a delay due to its association with both the timing of the mosquito life cycle and the incubation period of the virus. Different studies have provided varying evidence. For example, *Moirano* and colleagues (2018) reported a delay of 5-6 weeks between the increase in temperature and the rise of WNV human cases. In contrast, *Calzolari* and colleagues (2020) (25)reported a delay of 2 weeks, whereas *Groen* and colleagues (2017) examined the immediate effects of temperature, with no period. (21,51)

- **Precipitation.**

Results on the correlation between precipitation and WNV incidence are controversial: some studies have found a negative correlation, while others have found a positive one (47). Studies hypothesize that the **correlation between precipitation and WNV is area dependent**, as there is a <u>complex interaction between water precipitation and other environmental variables</u> in determining the impact on WNV epidemiology (51).

The presence of water is essential for adequate larval development habitat, as part of the mosquito life cycle is water dependent (26). However, excessive rainfall can damage habitats suitable for mosquito breeding, destroying egg rafts and killing larvae (54).

It was observed again that the influence of this climatic parameter varies with the season. Increased rainfall in late winter and early spring correlates with increased mosquito density and WNV incidence (47). The mosquito season is longer when rainfall starts in late spring, allowing the mosquito population to expand (55), while it starts later and lasts less when rainfall starts earlier (53). This condition occurs because, during the spring rainfall deficit phase, there is a concentration of vectors and reservoirs in the areas with the most water, with favorable interaction between species and, consequently, circulation of WNV between them (15,16).

There is a negative correlation between months with higher accumulated rainfall and WNV incidence, due to an effect on larvae and interaction with other factors (e.g., temperature, which is lower in months with higher rainfall). Mosquito densities are higher in months with low rainfall: excess rainfall has a negative effect on mosquito survival and ability to reproduce (47)

- **Relative Humidity (RH).**

RH is defined as the ratio of the water vapor pressure in a mass of air to its saturation at the same temperature. RH is expressed as a percentage.
Higher temperatures are often associated with lower relative humidity (RH) (21). Certain studies have reported a negative correlation between mosquito population density and RH: a reduction in RH results in an increase in mosquito population, owing to more arid atmospheric conditions. In the Po Valley and Emilia Romagna, the correlation reaches its peak with a delay of 3-4 weeks ((21). Similarly, to precipitation, outcomes for this parameter are debatable, and they vary depending on the specific study area (47).

- **Drought indices.**

Normalized Difference Vegetation Index (NDVI). NDVI is a measure of vegetation health that relates vegetation to its photosynthetic capacity. It is derived from remote sensing satellite data. The index is based on the difference between visible (RED) and near-infrared (NIR) sunlight reflected by plants. The calculation of NDVI is as follows: NDVI = (NIR - RED) / (NIR + RED). Lower NDVI values indicate drought because vegetation reduces its photosynthetic capacity under water stress (56). Several studies have shown a negative correlation between NDVI and mosquito density (47).

Normalized Difference Water Index (NDWI). The Normalized Difference Water Index (NDWI), which employs satellite imagery, is used to evaluate the water content of vegetation. As with the Normalized Difference Vegetation Index (NDVI), the NDWI is a drought index (57). Several studies have employed the NDWI parameter and noted a correlation between it and the onset and duration of the mosquito and WNV seasons (47).

Evapotranspiration. Evapotranspiration is the quantity of water lost to the atmosphere due to vegetation transpiration and direct evaporation from the surface, soil, and vegetation. It is an indicator of drought and has a positive correlation with vector density (47).

- **Seasonality**

Human infection with WNV is a seasonal infection that occurs close to the summer season, which is the most favorable time for the vectors. How temperature and rainfall influence the seasonality of mosquitoes and consequently the epidemiology of WNV has already been discussed. To conclude this section, it is worth highlighting the role of daylight: the shortening of the day regulates the diapause of adult mosquitoes, thus influencing the length of the mosquito season and the interruption of virus transmission (28,47).

Climatic parameters	Literature available evidence	Hypotheses in the literature (requiring further study)
Temperature	An increase in environmental mean temperature causes • **Direct effects** (on WNV infection and transmission): - increased mosquito infection rate (49). - decreased extrinsic incubation time (faster incubation) (24,50). - increased WNV replication rate in mosquitoes (51). - increased transmission rate from mosquitoes to hosts (49). • **Indirect effects** (on vector survival/abundance): - on the population: increased presence, abundance, and growth (24). - on feeding behaviour: increased biting rate and flight activity (20,27). - life cycle: optimum range for survival and dispersal: 25°C to 35°C (46). A reduction in environmental mean temperature correlates with a significant reduction in the mosquito population (52).	An **increase in spring temperature** above the seasonal average correlates with an increase in mosquito population density and a prolongation of the mosquito season (38,46,52). An increase in temperature does not necessarily correlate with an increase in mosquito abundance: this seems to be explained by the fact that an increase in environmental mean temperature above average correlates with an increase in adult mosquito mortality (27).
Precipitation	Correlations with rainfall are controversial and **highly area dependent**, reflecting the complex interactions between different climatic and environmental variables (47). A wet environment is crucial for the development of mosquitoes (25). Excessive rainfall can damage mosquito breeding habitats. Low rainfall correlates with an increase in population density (46). Concentrated rainfall in late spring/early summer favours expansion and increase in population and mosquito season (54).	A **spring characterised by lower rainfall** (and above-average temperatures) favours the formation of ecological niches in which the enzootic cycle between mosquitoes and birds occurs, leading to WNV amplification. The presence of rain in late spring/early summer then favours the expansion of the WNV infected population (38,46,52).
Relative Humidity	Correlations with RH are **highly area dependent (20).**	Some studies have reported that reduction in RH correlates in an increase in mosquito population (46).
Drought indices	-	Correlations with NDWI, NDVI and evapotranspiration are not completely understood and controversial (46)
Seasonality and Daylight	Summer season is the season most represented by mosquito circulation, and consequently, by WNV infections. The shortening of the day regulates diapause of adult mosquitoes, influencing the length of mosquito season (27,46)	-

Figure 5, Summary table of the main climatic variables studied in correlation to WNV epidemiology.

Environmental influence

As mentioned earlier, the way climatic factors interact with the environmental characteristics of an area is complex: the same climatic event can have different impacts on the epidemiology of West Nile Virus (WNV) depending on the area's specific features. *Ippoliti* and colleagues (2019) combined environmental and climatic characteristics and divided Italy into eco-regions. The authors observed that the areas with high WNV detection have similar ecoregional characteristics. Therefore, areas with similar eco-regionalization to those in which the virus is circulating can be potential risk areas for WNV (12).

Several environmental factors influence the epidemiology of WNV. The **presence of wetlands and marshes** is particularly important (47). As previously mentioned, the mosquito's life cycle occurs partly in an aquatic environment, and the existence of stagnant water pools is a crucial condition for the breeding of larvae and the subsequent emergence of vectors ((26). Nevertheless, man can also artificially create areas suitable for mosquitoes, as in the case of **agricultural land practices like rice paddies or irrigation** (47). The occurrence of drought often leads to the presence of human-caused activities that create such areas. In the absence of rainfall, it promotes the creation of ecological niches mentioned earlier. These niches are where vectors and reservoirs interact, resulting in a high degree of viral amplification (13).

The **proximity of** these **wetlands to inhabited areas** is an important consideration for human WNV infection. **Land use and urbanization** affect the interaction of mosquitoes with blood meal sources such as birds and dead-end hosts (e.g., humans or horses), due to their limited flight range (47). Studying how humans interact with mosquito habitats is crucial as there may be interaction, complete overlap or no relationship between human habitats and ecological niches in which mosquitoes develop. This variation in circulation of West Nile Virus (WNV) and number of human infections in areas of similar eco-regionalization could be a result of these interactions (12). The epidemiology of WNV can be better understood by studying the differences between **rural and urban area**s in high viral circulation regions (58). However, estimating this relationship is difficult because WNV infection and WNND data are collected and aggregated by province and there is limited research on this aspect in the

literature. Based on a study conducted in Emilia Romagna, in this area there seems to be no difference in infection between rural and urban areas because there is an overlap between human and vectors' habitats (39,53).
Finally, also **altitude** is an environmental parameter that closely correlates with climate. Lower altitudes are positively associated with the spread of WNV. This is because higher altitude more frequently experiences temperatures that are not favorable for mosquito development (47).

Climate Change and Arboviroses as a One Health Challenge.

As illustrated in the prior sections, the epidemiology of WNV is significantly affected by climate. In the current circumstances, with the presence of a fluctuating climate, it is rational to assume that climate change has already had an impact on epidemiology and is still influencing and will continue to influence the epidemiology of WNV and other arboviruses in the years to come (3,46).

In recent years, Northern Italy and Lombardy have experienced rising temperatures and an increase in heat wave and exceptionally hot day occurrence (59). During the period 2021-2025, temperatures are projected to rise by a margin of +1.5°C compared to 1961-1990, with averages of +2°C in summer and +1°C in winter. However, some cities, such as Milan, may see more significant increases. These trends could escalate to +5°C without implementation of effective climate change adaptation strategies.
This is also linked to reduced precipitation, particularly in spring, where it is estimated to drop by 9% within a century (60).

The effects of climate change on health are widely acknowledged and will undoubtedly increase. The spread of arboviruses is just one facet of a broader problem(61). According to the World Health Organization (WHO), the climate crisis is a current health crisis (62). As a result, there will be a significant increase in mortality rates from cardiovascular disease associated with heat waves, as well as health problems associated with extreme weather events. This will inevitably put tremendous strain on healthcare systems, especially the less advanced ones in the poorest settings (10).

In addition to West Nile virus, which has been endemic in Europe for fifteen years, new arboviruses are emerging. Examples of this are the endemic Dengue cases recorded during the summer of 2023 in Italy (63,64), as well as the cases of native Chikungunya recorded in 2007 in Emilia Romagna and in 2017 in Lazio and Calabria (67). Although the relationship between climate and arboviruses such as WNV has received considerable attention in academic literature, new studies are needed to strengthen the scientific evidence and build future predictive models.

The *One Health* approach is now widely acknowledged as crucial for arbovirus surveillance. As noted previously, the detection of WNV circulation calls for a collaborative effort between clinicians, veterinarians, entomologists, public health specialists, and political authorities. The *One Health* approach is also essential for predicting the future course of arboviruses (44). For instance, various research groups in Europe have developed predictive mathematical models using this approach (49,65,66). In other words, arboviruses present a challenge to the *One Health* approach, providing an occasion to demonstrate its efficiency and reliability.

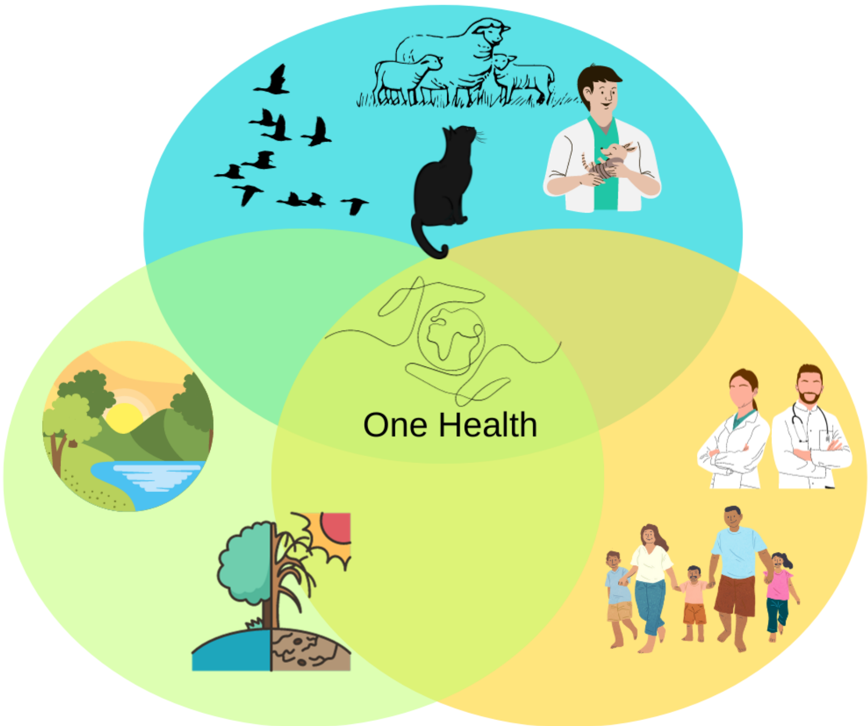

Figure 6, One Health Approach

Chapter II: Objective, Materials and Methods

TYPE AND OBJECTIVE OF THE STUDY

This research is a retrospective observational study that aims to investigate the epidemiology of WNV infection in Lombardy between 2013 and 2022 through a One Health approach. The primary goal is to analyze the effect of climate factors such as temperature and cumulative rainfall, and their correlation with WNV spread (both mosquitoes' and humans' infections). These observations will enable the assessment of any association between climatic events and the incidence of human cases of WNV infection, including asymptomatic donors, WNF, and WNND.

To our knowledge, this study is the first to investigate the relationship between WNV infection records and climate data in Lombardy. Moreover, assessing the descriptive epidemiology of WNV in Lombardy during the period of 2013-2022 served as a secondary objective of this research.

In the current public health context, as we already stated, we are witnessing the emergence of arboviruses that were once alien. Studying the evolving climate is a crucial aspect of predicting the future of arboviral diseases in Europe (48).

MATERIALS

Literature review

We undertook a thorough search of the literature to identify peer-reviewed articles published between 1998 and 2023 that are relevant for this research. We used PubMed and Embase. Additional articles were identified from the retrieved articles' references. The literature was last checked on September 15, 2023. The keywords used include West Nile virus or West Nile Fever or West Nile Neuroinvasive Disease or West Nile Virus Infection and climate change or climate condition or climatology or temperature related phenomena and Italy; West Nile Virus or West Nile virus or West Nile Fever or West Nile Neuroinvasive Disease or West Nile Virus Infection and mosquito or culex or aedes and Italy.

After a rigorous literature review, the variables needed for the study were identified categorized in 3 orders: human, entomological, and environmental. They will be the subject of discussion below as a whole.

Human data

In order to study the correlation between human cases of WNV infection and climate, we retrieved from the records stored in the Italian Arboviroses national platform the history of all cases of human WNV infection recorded in Lombardy during the study period.

To help clarify, let us recall the definition of human case of WNV infection, which is established by clinical and laboratory criteria.

The **clinical criteria** include individuals who present with fever or one of the following clinical manifestations: encephalitis, cerebrospinal fluid (CSF) meningitis, polyradiculitis-neuritis (similar to Guillain-Barré), or acute flaccid paralysis (68).

The **laboratory criteria** are divided into two categories: those for **probable cases,** including a specific IgM antibody response to WNV in serum, and those for **confirmed cases**, consisting of positivity for WNV isolation in serum, urine and/or CSF; detection of WNV nucleic acid in blood, urine and/or CSF; specific antibody response to WNV (IgM) in CSF; high titer of WNV IgM; and proof of WNV IgG in serum, as confirmed by neutralization (68).

A person who meets the clinical and laboratory criteria for probable case is defined as a probable case of WNV infection. On the other hand, an individual who meets at least one of the laboratory criteria for a confirmed case is defined as a WNV infection confirmed case (68).

Possible cases were previously classified as patients exhibiting clinical symptoms but not meeting laboratory criteria. However, this classification is no longer in use (68,69).

For each patient, the following variables were collected:

- Sex: male or female.
- Age at reporting, categorized by 10-year intervals.
- Diagnostic tests conducted to identify cases of WNV, whether positive or negative, and date: serology, CSF and/or urine.
- Date on which the serology was carried out.
- Case classification: asymptomatic confirmed, asymptomatic probable, confirmed donor, probable donor, confirmed with fever, probable with fever, confirmed neuroinvasive, probable neuroinvasive, confirmed symptomatic, probable symptomatic, possible, not classified.
- Imported or autochthonous case.
- Province of Exposure: Province where the patient was likely exposed.
- Province of Residence: Province where the patient resided.
- Patient outcome: deceased, severe, cured, improving, not applicable, or unknown.
- Patient follow-up outcome: deceased, cured, improving, or not known.
- Date of report to Italian national Arboviroses platform, and last update.

In Italy, it is mandatory to report all human cases of WNV infection to the Istituto Superiore di Sanità (ISS) in accordance with the Italian National Plan for Prevention, Surveillance and Response to Arboviruses (PNA) 2020-2025 (and corresponding WNV surveillance plans preceding the PNA) (8). Such reports are submitted through the national platform https://arbo.iss.it/Default.aspx.

To acquire such information, we sought assistance from Region Lombardy. We specifically requested data from the Prevention Organizational Unit (UOP). The UOP oversees the coordination and planning of prevention measures to promote public health, control the spread of diseases, and guarantee the safety of food within the region. All its activities are conducted in collaboration with the Italian Ministry of Health. The UOP monitors cases of WNV infections and human infections in cooperation with Istituto Zooprofilattico Sperimentale of Lombardy and Emilia

Romagna (IZSLER) and can access the national platform from which procured us the data requested for the study (70).

For our study, we used the date of serology as the date of the case, even if some data were missing. This is because case reporting to the ISS is not immediate and the interval between patient positivity and reporting to the platform is variable. If we want to correlate human WNV cases with climate, the serological date corresponds to the date of diagnosis and is the one that correlates best with climate assessments.

Entomological data

As stated earlier in chapter one, climatic effects on the human epidemiology of WNV are influenced by climate's impact on mosquitoes. Mosquitoes are susceptible to temperature and precipitation variations, which ultimately determine the suitability of their environment for both development and virus transmission. The vectors are specifically impacted by changes in climate in terms of abundance, density, and infection rate (47).

The data we used were the dates of the first detection of WNV in each mosquito trap installed in the Lombardy region, year by year. The presence of positive traps indicates the positivity of the mosquito population in the defined area, which represents the potential infectious risk to humans, as mosquitoes are vectors of WNV.

We acquired the annual record of trap activations and corresponding dates via the Istituto IZSLER platform accessible at the following web address https://archive.izsler.it/pls/izs_bs/v3_s2ew_consultazione.mostra_pagina?id_pagina=828.

The Istituto Zooprofilattico Sperimentale of Lombardy and Emilia Romagna (IZSLER) is a public health body with management, technical and administrative autonomy operating within the wider Italian National Health Service. The Istituti Zooprofilattici Sperimentali (Experimental Zooprophylactic Institutes) provide epidemiological surveillance, experimental research, staff training, laboratory support and diagnostics in the field of official food control (71).

In the area of WNV infection, they are responsible for territorial, entomological, and vertebrate epidemiological surveillance. Surveillance in vertebrates is carried out in farmed equines showing symptoms consistent with WNV infection and in avian reservoirs, where both active and passive surveillance is conducted (71).

Entomological surveillance is carried out by setting traps in the territory at risk (one trap every 20 square kilometers), which are checked every two weeks from 1 June. CO2-CDC mosquito traps are devices used to trap and monitor mosquitoes. They work by emitting carbon dioxide (CO_2) and other attractants to lure mosquitoes. A fan creates an air current that traps them inside the trap. The captured mosquitoes are then identified and tested for potential pathogens. These traps are essential for epidemiological surveillance to monitor and prevent mosquito-borne diseases.

Figure 7, Lombardy region, quadrant subdivision for mosquito traps, last update (2022). The + symbol represents where mosquito traps are placed (73).

Surveillance on individual traps is stopped if the trap is positive for WNV: from that moment, the province in which the trap is located is identified as having active virus circulation and systematic screening of blood and tissue donors using NAT tests is triggered to avoid infected transfusions (72).

Over the course of 10 years, IZSLER's trap placement strategy underwent significant changes. In 2013, 30 traps were positioned only in the most at-risk areas, equivalent to the Po Valley, in a dispersed manner. In 2014, the same 30 traps were more systematically divided: the defined risk area was divided into 30 quadrants of 20 square kilometers each, and a mosquito trap was placed in each quadrant. By 2015, the number of traps had increased to 38, with detection also expanded to provinces such as Como, Lecco, and Sondrio, which were deemed to be at lower risk of WNV circulation. Between 2016 and 2019, there were 40 quadrants of detection; this number rose to 43 by 2022 due to the installation of 3 additional traps in the province of Sondrio (72).

To assess the effectiveness of the traps, we collected yearly positivity data, which we then aggregated by week and province due to the limited number of traps. This provided us with weekly information on the number of traps in each province that tested positive.

Climate data

Based on the literature, **temperature** and **precipitation** were found to be the parameters most strongly correlated with WNV epidemiology.

Therefore, **daily mean temperature** and **daily cumulative precipitation** were selected as study variables.

In weather and climate models, mean temperature represents the atmospheric air temperature at 2 meters above the surface during a given period (73). The cumulated precipitation over a period (mm/period) corresponds to the water equivalent flux - i.e., rain or snow - reaching the land surface, expressed as $kg \cdot m^{-2} \cdot sec^{-2}$ and converted to mm/day (74).

Daily collection of temperature and cumulative rainfall data, from daily 30 arc second resolution rainfall and temperature fields across the study area since the 1960s, was carried out. These data were made available by the Department of Environmental Sciences and Policies of the University of Milan directed by Professor Maurizio Maugeri, president of the master's degree Course Environmental Change and Global Sustainability at the University of Milan. To obtain climatic values for each province, identical coordinates to those of the IZSLER mosquito trap placements for that year were used as reference. Subsequently, a value was obtained for each coordinate by dividing the Lombardy territory into quadrants of 20 square meters, maintaining IZSLER structure.
Daily maximum and minimum temperature readings were obtained for each coordinate, covering the period between 1 January 2013 and 31 January 2022.
The temperature data were compiled by province. This provided a clear and objective overview of the temperature trends.
Similarly, employing the above system, the daily cumulative rainfall value from 1st January 2013 until 31st August 2020 was extracted for each coordinate.
Daily cumulative rainfall data from 1 September 2020 to 31 January 2022 were acquired via the Agenzia Regionale per la Protezione dell'Ambiente (ARPA) - Lombardia. Unfortunately, the location of the ARPA control units does not precisely align with the positions of the IZSLER mosquito traps. Consequently, the ARPA control units in closest proximity to the IZSLER coordinates, located within a 20 square kilometer radius of the trap, were utilized as reference points for the absent cumulative precipitation data.
As for the temperature, precipitation data were collated by province.

ARPA Lombardia is a regional agency in Lombardy, Italy, responsible for monitoring environmental and public health. Data on air, water, and soil quality are systematically collected, along with meteorological information such as temperature, precipitation, and wind. The agency assesses environmental risks, regulates industrial emissions, manages environmental emergencies, conducts scientific research, and disseminates information about environmental protection and public health to the relevant authorities and the public (75).

Using the https://www.arpalombardia.it/temi-ambientali/meteo-e-clima/form-richiesta-dati/ platform, it was possible to request and obtain the relevant cumulative rainfall data. As in the previous period, a survey point was chosen for each quadrant used for the traps (one control unit for each quadrant).

STATISTICAL ANALYSIS

Regarding the section on descriptive epidemiology, statistical analyses included descriptive statistics as absolute and relative frequencies for categorical factors, for continuous variables data have been reconducted to specific range class and described also as absolute and relative frequencies.
The statistical analyses and graphs were generated using statistical software packages including Statistical Package for Social Science (SPSS) - Statistics for Data Analysis version 28 and Microsoft Excel 365.

The retrospective observational study on the correlation between WNV and climate focused on 5 provinces that were significantly affected by WNV during the decade of the study: virus during the decade under review: Brescia, Cremona, Lodi, Mantua and Milan. For every year of study, a scatterplot was created for each of these provinces, with the time interval being represented on the x-axis. Moreover, this time interval corresponds to the period from April to October, the season during which the spread of mosquitoes and West Nile Virus is commonly observed. The graph depicts various time series (Tmax, Tmin, Cumulative PCP), shown as lines on the y-axis. Cases involving humans and positive readings from traps have been marked as dots on the graphics.

This study was carried out in accordance with current ethical and legal regulations without necessitating approval from an ethics committee, as all human data collected and analyzed were anonymized.

Chapter III: Results

Descriptive Epidemiological Study

Between 2013 and 2022, there were 428 officially reported cases of WNV infection in Lombardy.

During the study period 110 patients were female (equivalent to 25.7% of the total) and 318 patients were male (accounting for 74.3% of the total). These data indicate that incidence of infection is higher among males (about three quarters of the total cases recorded).

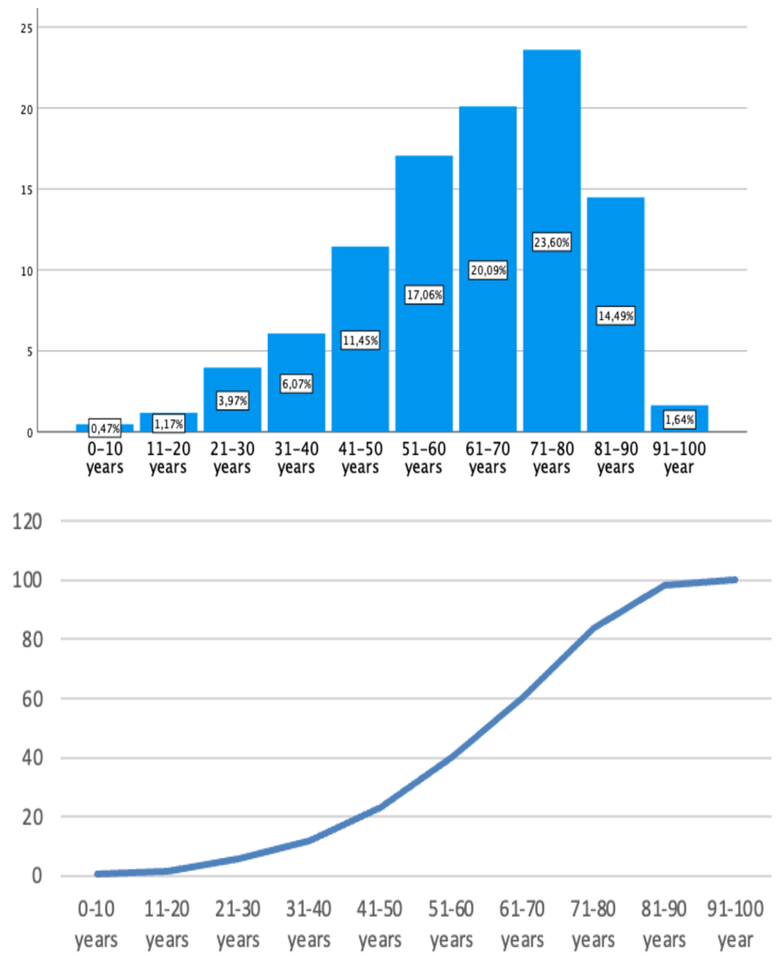

Figure 8. Distribution of WNV cases in different age groups in Lombardy (2013-2022). The lower graph shows their cumulative distribution.

Figure 8 shows the **distribution of the population by age groups** (ten-year intervals). The two age groups that were most affected in terms of absolute numbers were those between 71 and 80 years of age (23.6% of cases) and between 61 and 70 years of age

(20.1% of cases). Moreover, a cumulative analysis of the data reveals that about three quarters (76.9%) of cases occurred in individuals aged between 51 and 100 years.

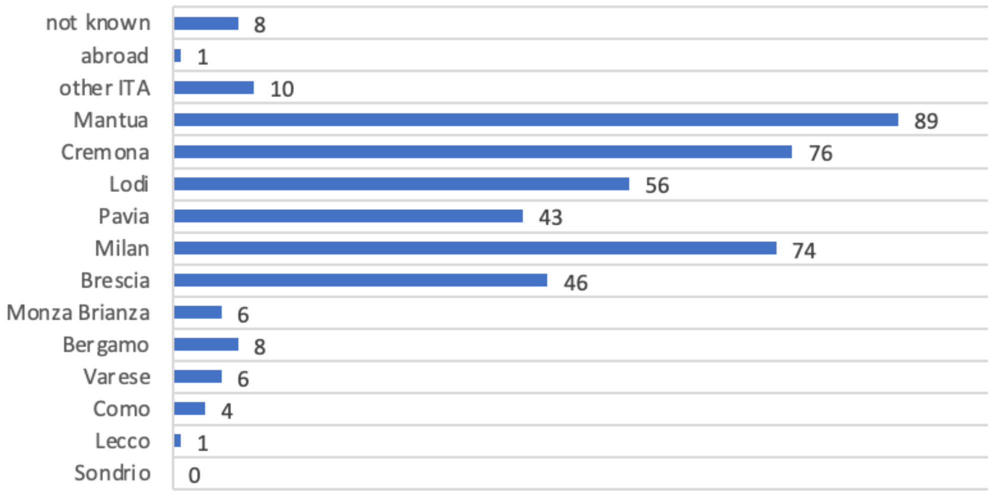

Figure 9, Distribution of WNV cases by Province in Lombardy, 2013-2022

Figure 9 illustrates that the top 3 provinces with the highest number of cases in Lombardy between 2013 and 2022 have been Mantua (20.8% of cases), Cremona (17.8% of cases) and Milan (17.3% of cases), followed by Lodi (13.1%) and Brescia (10.7%).

This confirms that the provinces most affected in Lombardy are those situated in the Po Valley.

Moreover, there were eleven cases of exposure outside Lombardy, accounting for 2.6% of the total cases (1 case in Asti, Bolzano, Verona, and the province of Venice each, 4 cases from Piacenza, 2 cases from the province of Verbano-Cusio-Ossola, and 1 case abroad).

However, the province of exposure for 8 cases, which comprised 1.9% of the total cases, was not identifiable.

Of the 428 cases examined in the study, 413 were deemed autochthonous while only 5 cases were attributed to imported infection. The autochthonous or imported status of the remaining 10 cases is uncertain. This demonstrates that WNV is now established as an endemic disease in Lombardy.

Total	Case type	Clinical classification	Category	FREQUENCY	PERCENTAGE OF TOTAL	% EXCLUDING NON CLINICAL CASES
TOTAL RECORDED CASES: 428	CONFIRMED CASES: 319 74,5%	CLINICAL CLASSIFICATION: 244 76,5%	DONORS	104	32,6%	42,6%
TOTAL RECORDED CASES: 428	CONFIRMED CASES: 319 74,5%	CLINICAL CLASSIFICATION: 244 76,5%	ASYMPTOMATIC	6	1,9%	2,5%
TOTAL RECORDED CASES: 428	CONFIRMED CASES: 319 74,5%	CLINICAL CLASSIFICATION: 244 76,5%	SYMPTOMATIC	3	0,9%	1,2%
TOTAL RECORDED CASES: 428	CONFIRMED CASES: 319 74,5%	CLINICAL CLASSIFICATION: 244 76,5%	FEVER	45	14,1%	18,4%
TOTAL RECORDED CASES: 428	CONFIRMED CASES: 319 74,5%	CLINICAL CLASSIFICATION: 244 76,5%	NEUROINVASIVE	86	27%	35,2%
TOTAL RECORDED CASES: 428	CONFIRMED CASES: 319 74,5%	CONFIRMED WITHOUT CLINICAL CLASSIFICATION		75	23,5%	-
TOTAL RECORDED CASES: 428	PROBABLE CASES: 73 17,1%	CLINICAL CLASSIFICATION: 62 84,9%	DONORS	9	12,3%	14,5%
TOTAL RECORDED CASES: 428	PROBABLE CASES: 73 17,1%	CLINICAL CLASSIFICATION: 62 84,9%	ASYMPTOMATIC	6	8,2%	9,7%
TOTAL RECORDED CASES: 428	PROBABLE CASES: 73 17,1%	CLINICAL CLASSIFICATION: 62 84,9%	SYMPTOMATIC	2	2,7%	3,2%
TOTAL RECORDED CASES: 428	PROBABLE CASES: 73 17,1%	CLINICAL CLASSIFICATION: 62 84,9%	FEVER	24	32,9%	38,7%
TOTAL RECORDED CASES: 428	PROBABLE CASES: 73 17,1%	CLINICAL CLASSIFICATION: 62 84,9%	NEUROINVASIVE	21	28,8%	33,9%
TOTAL RECORDED CASES: 428	PROBABLE CASES: 73 17,1%	PROBABLES WITHOUT CLINICAL CLASSIFICATION		11	15,1%	-
TOTAL RECORDED CASES: 428	OTHER: 36 8,4%		POSSIBLE	2	-	-
TOTAL RECORDED CASES: 428	OTHER: 36 8,4%		UNCLASSIFIED	34	-	-

Figure 10, Record WNV cases by classification: confirmed, probable and other.

Over the 10-year period under study, there were a total of 319 confirmed WNV cases, 73 probable cases, and 36 WNV cases classified as possible or unclassified.

Confirmed and probable cases were categorized based on their clinical characteristics as asymptomatic, donors, febrile (WNF), neuroinvasive (WNND), and symptomatic. It is noteworthy that clinical classification has only been introduced systematically since 2019. This explains why some recorded cases were only classified as confirmed or probable between these two categories. 75 confirmed and 11 probable cases were identified without clinical specification.

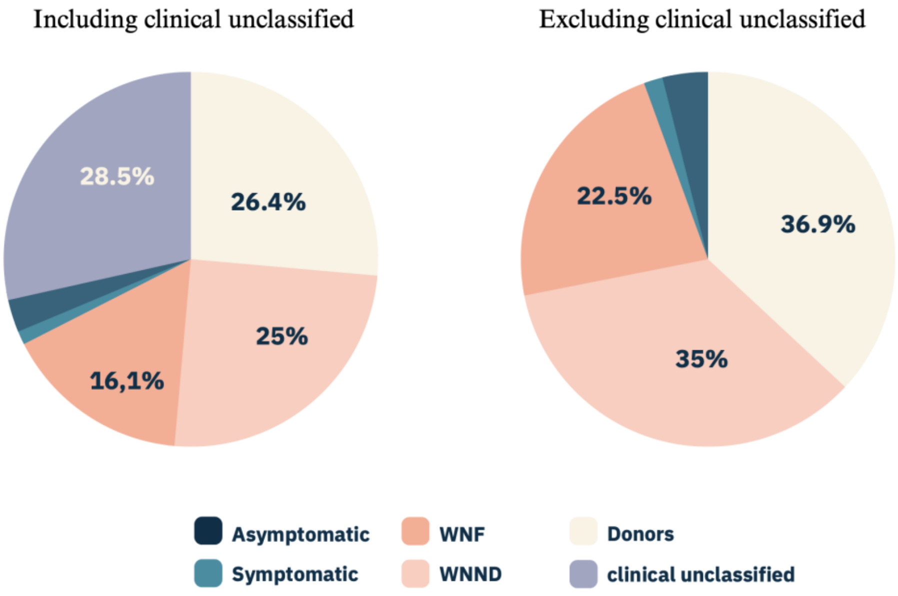

Figure 11, Distribution of WNV infection by clinical classification, combining confirmed and probable cases.

The figure above displays the distribution of cases according to clinical classification, encompassing both confirmed and presumed cases. The graph on the left shows that 28,5% of the 482 total cases lack clinical categorization.

The graph on the right omits cases not possessing a clinical classification, presenting subdivision amongst cases by clinic. It should be noted that within Lombardy, 36,9% of diagnoses pertain to donors, 35% concern WNND cases, while the remaining percentage is split between WNF (22,5%), symptomatic (2%), and asymptomatic (4%).

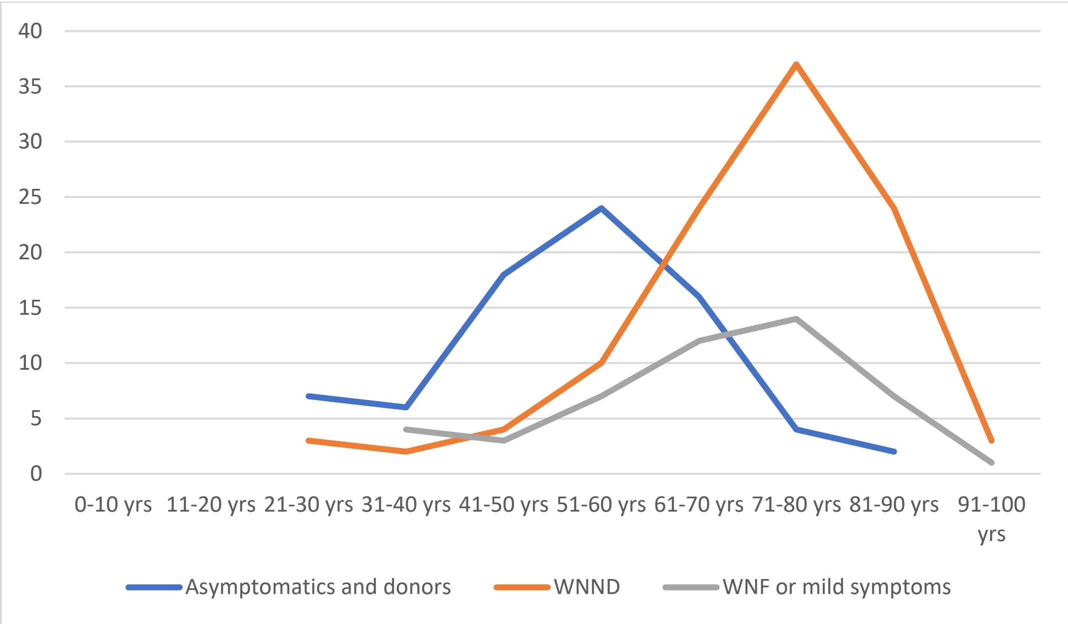

Figure 12 distribution of WNV human cases based on clinical classification and age (2019-2022)

During the period of 2019 to 2022, when clinical classification became more systematic, we reconstructed the distribution of cases based on clinical classification and age. In total, 251 cases were recorded over 4 years and grouped into three categories: asymptomatic, which included donors and cases classified as asymptomatic; WNF or mild symptoms, comprising cases classified as symptomatic but not specified further; and WNND. There were 18 cases that eluded clinical classification during this period. The graph above illustrates that cases of WNND and WNF/mild symptoms are concentrated among individuals over 60 years of age, whereas asymptomatic cases, primarily from donors, peak among individuals between 51 and 60 years of age. Therefore, it can be concluded that in Region Lombardy individuals under 60 years of age are predominantly affected by mild asymptomatic infection, while both mild and severe symptomatic cases are more evenly distributed among the population over 60 years of age.

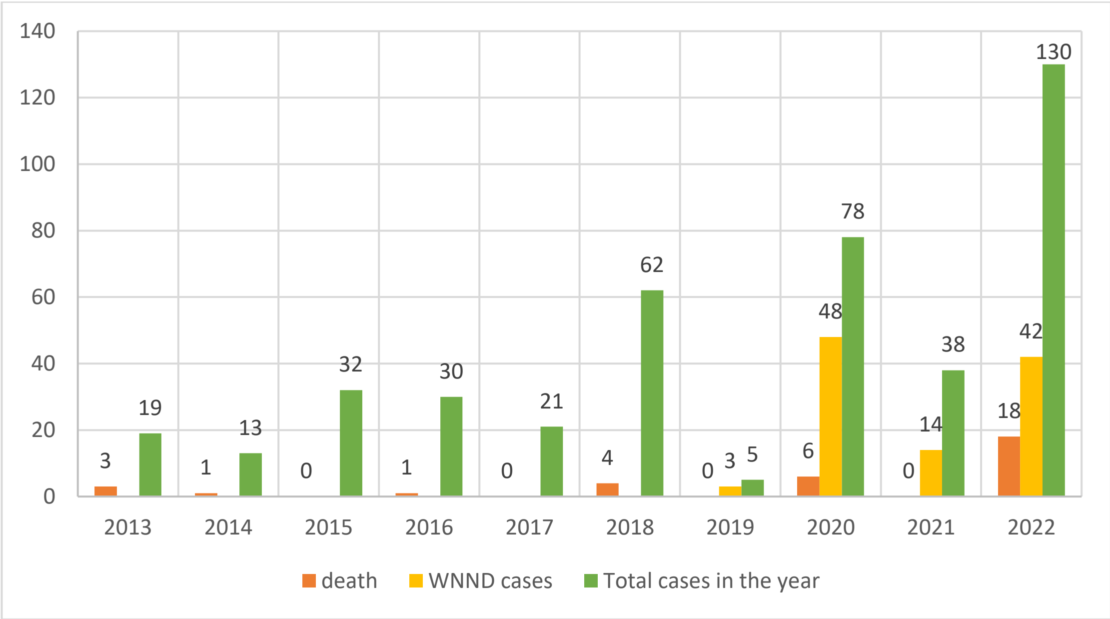

Figure 13, WNV epidemiology over the study period (2013-2022): total cases, WNND cases and patient deaths per year

To complete the epidemiological assessments, we looked at how the cases tended to occur over the years. The accompanying graph (green column) shows that the years with the highest incidence of WNV cases recorded in Lombardy were, in order, 2022 (130 cases), 2020 (78 cases) and 2018 (62 cases).

The orange column shows the deaths recorded over the years (33 in total). It should be noted that, according to our data, the outcome of 121 out of the 428 cases recorded is unknown (*Figure 14*). Therefore, there is uncertainty about the total number of deaths in Lombardy during the study period.

The yellow column represents the recorded WNND cases. They have been available only since 2019, the year when the clinical classification was introduced.

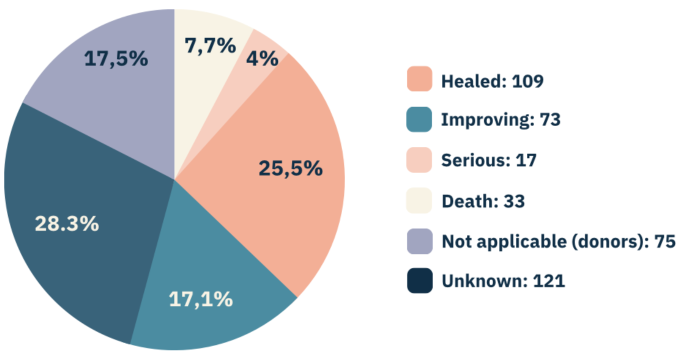

Figure 14, Classification by outcome and outcome follow up.

Figure 14 shows the classification of outcomes. As mentioned above and noted in the discussion of *Figure 13*, outcomes and follow-up are not systematically reported. It should be noted that in the case of blood donor patients, starting in 2019 the system has excluded the definition of outcome for these patients, which is therefore not applicable.

As can be seen from the table, in addition to death or cure, outcomes also include whether the patient is critically ill or recovering.

Inferential study to assess the correlation between climatic variables (cumulative rainfall, maximum temperature, minimum temperature) and WNV infection.

The following pages show graphs of temperature and precipitation trends in the provinces of Brescia (*Figures 16-18*), Cremona (*Figures 19-21*), Lodi (*Figures 22-24*), Mantua (*Figures 25-27*) and Milan (*Figures 28-30*). These provinces were responsible for about 80% of all cases in the Region during the study period (*Figure 9*).

The maximum temperature trend is shown in red, the minimum temperature in blue and the cumulative rainfall in green. The dots represent the human cases and the triangles the positive results of the mosquito traps.

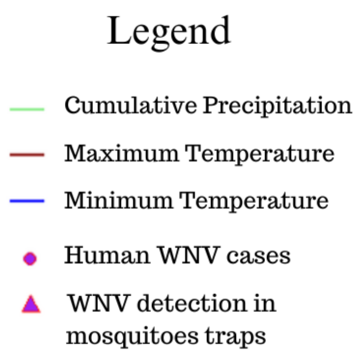

Figure 15, Legend of the following graphs

The results are discussed province by province after graphs.

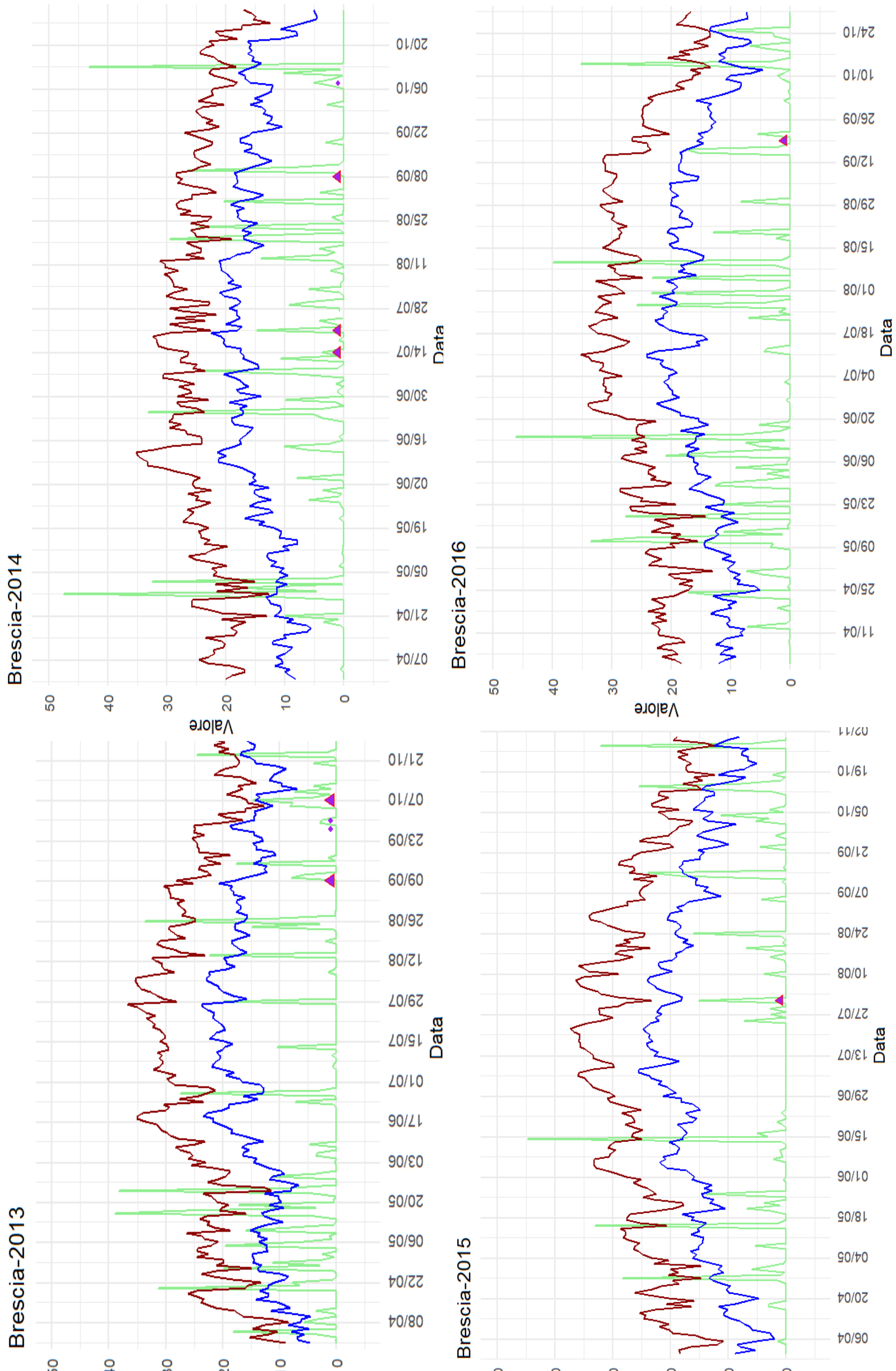

Figure 16, WNV, temperature and precipitation in Brescia, 2013.-2016

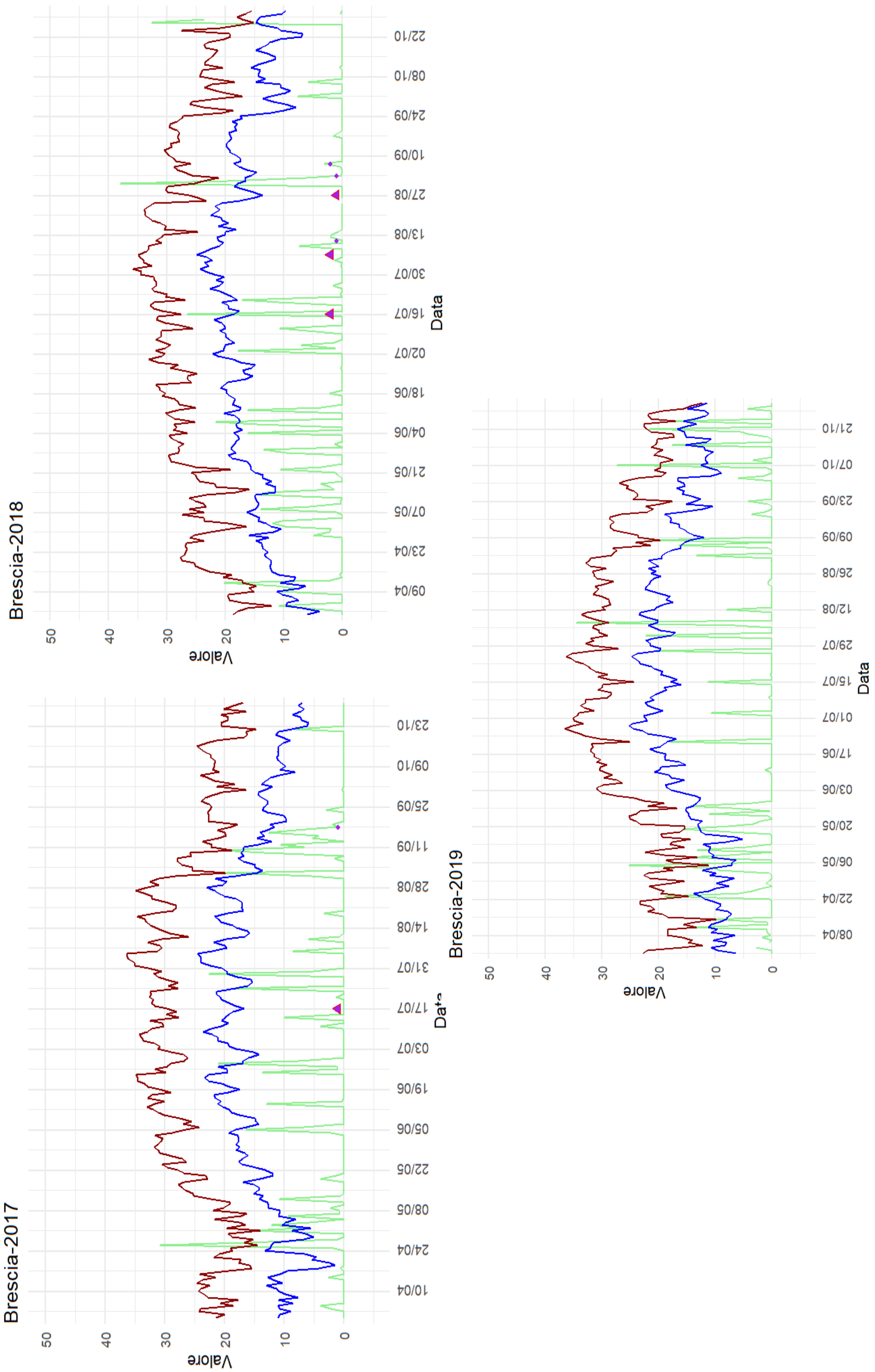

Figure 17, WNV, temperature and precipitation in Brescia, 2017-2019

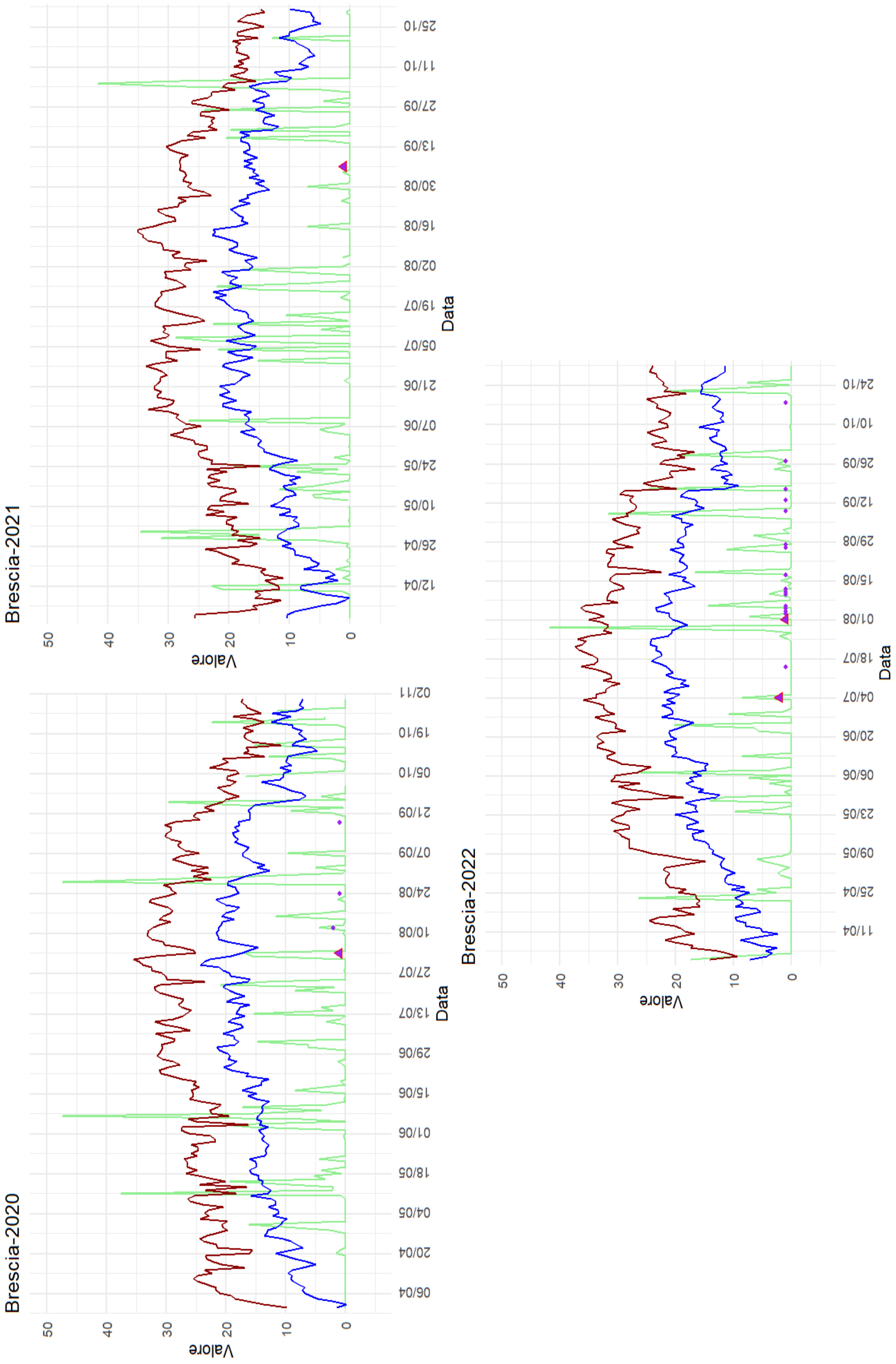

Figure 18, WNV, temperature and precipitation in Brescia, 2020-2022

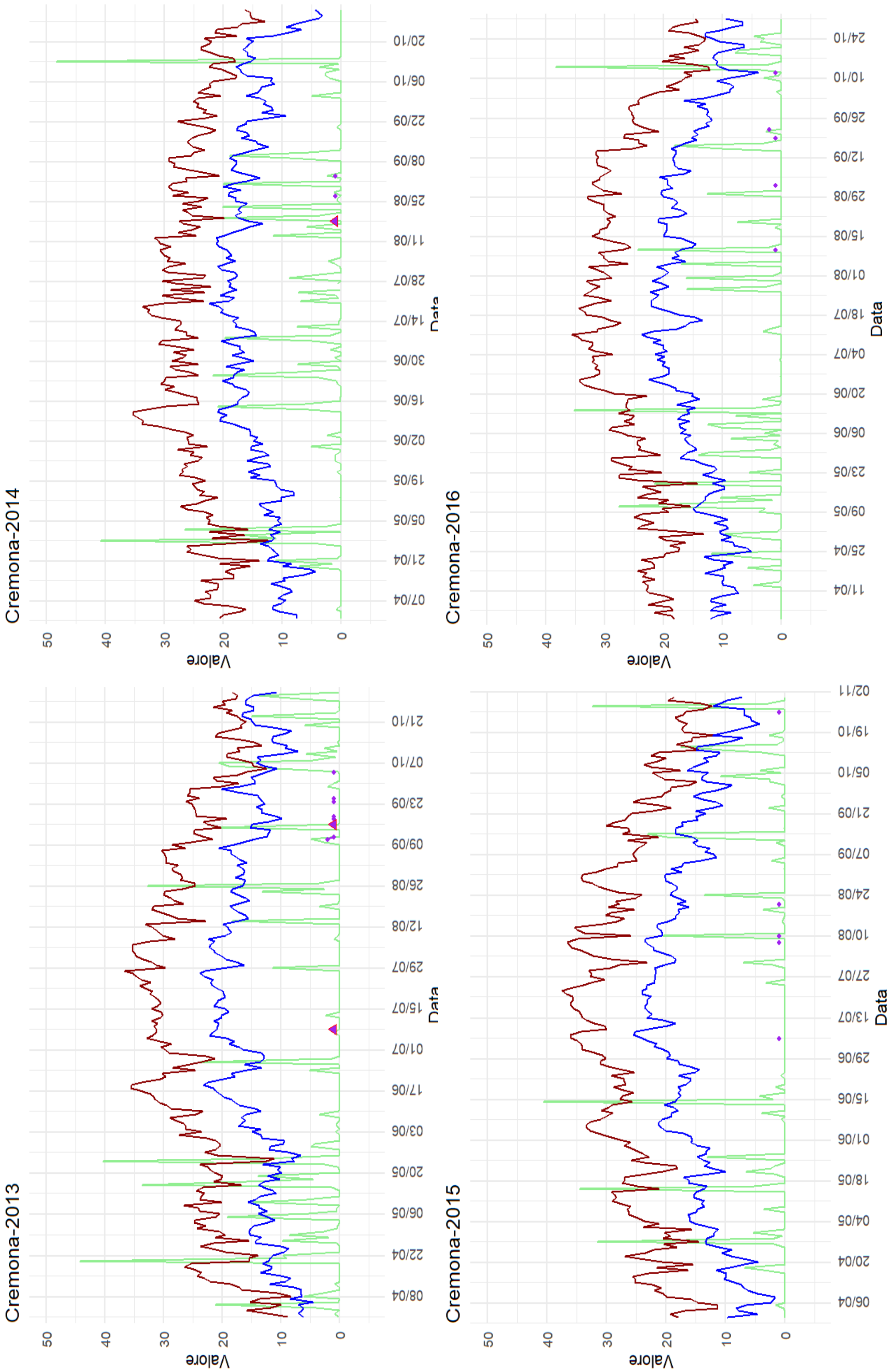

Figure 19, WNV, temperature and precipitation in Cremona, 2013-2016

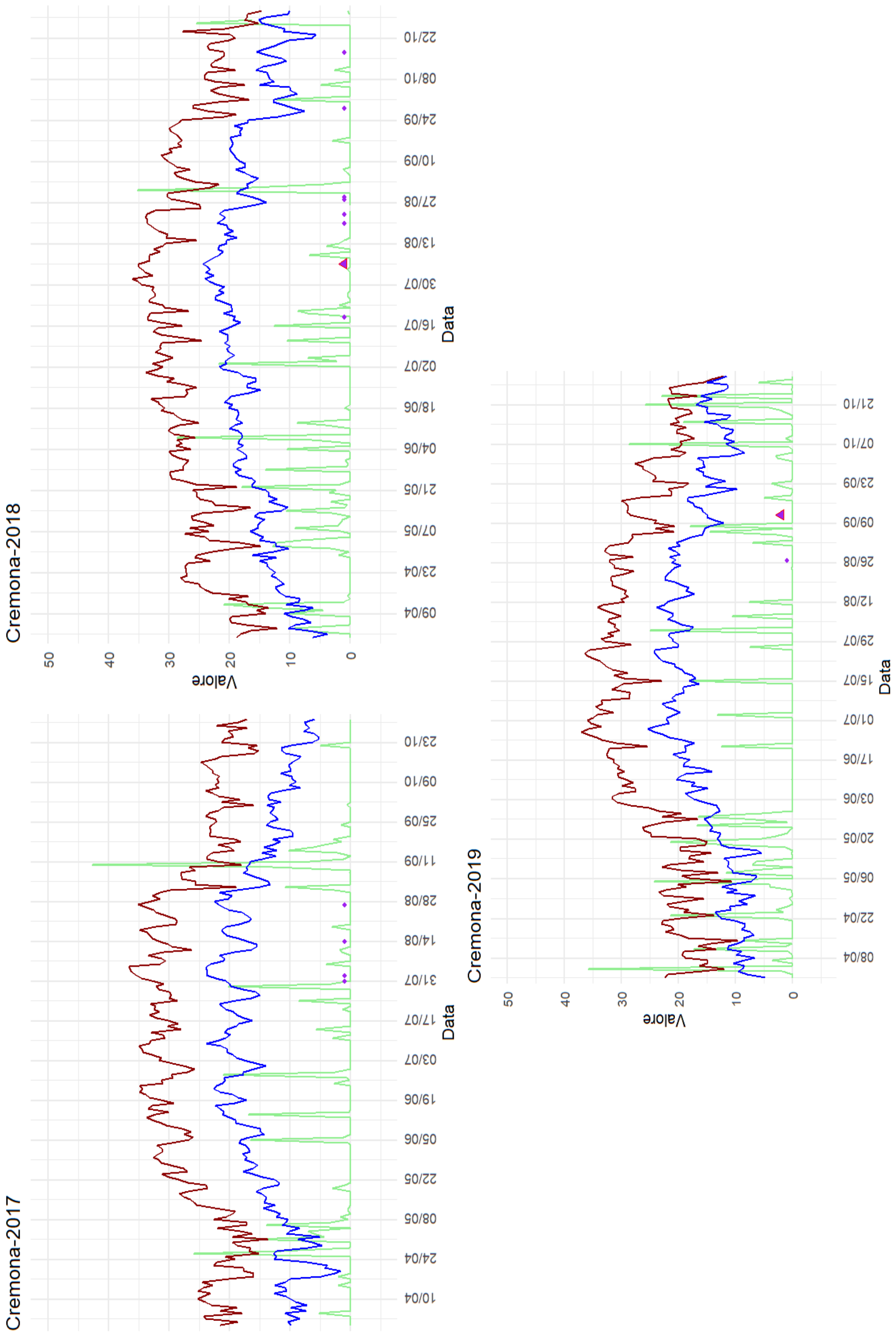

Figure 20, WNV, temperature and precipitation in Cremona, 2017-2019

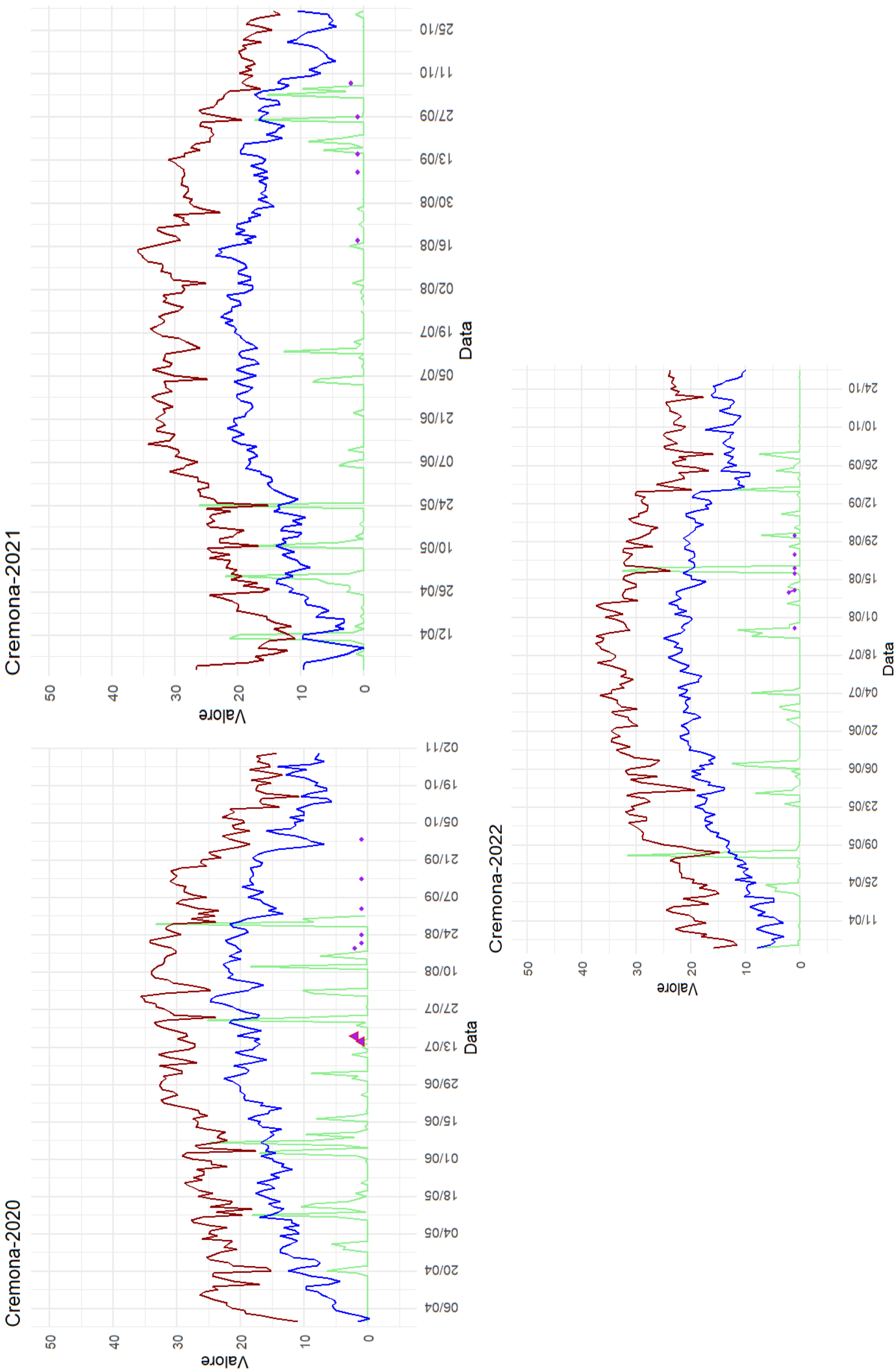

Figure 21, WNV, temperature and precipitation in Cremona, 2020-2022

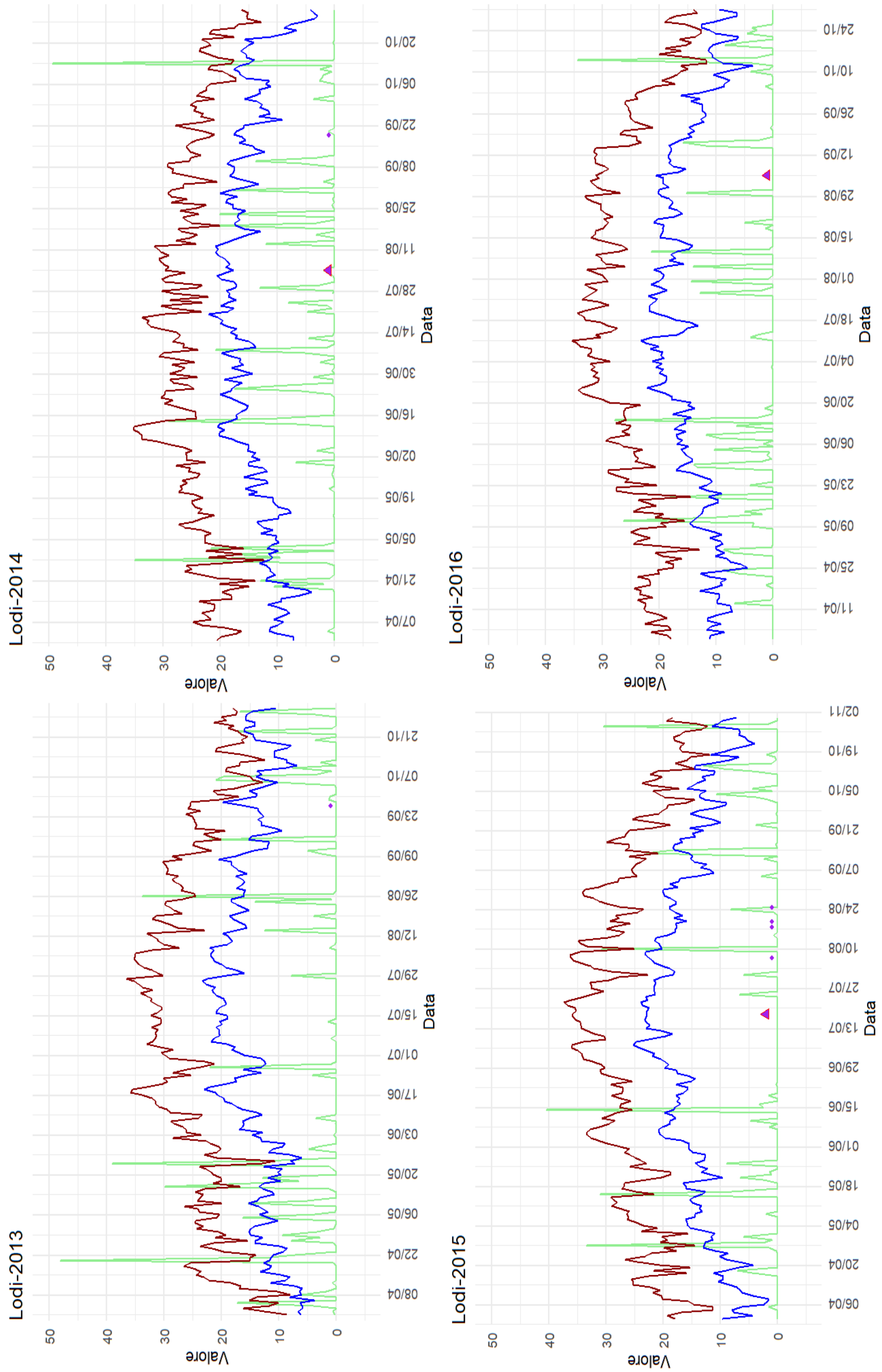

Figure 22, WNV, temperature and precipitation in Lodi, 2013-2016

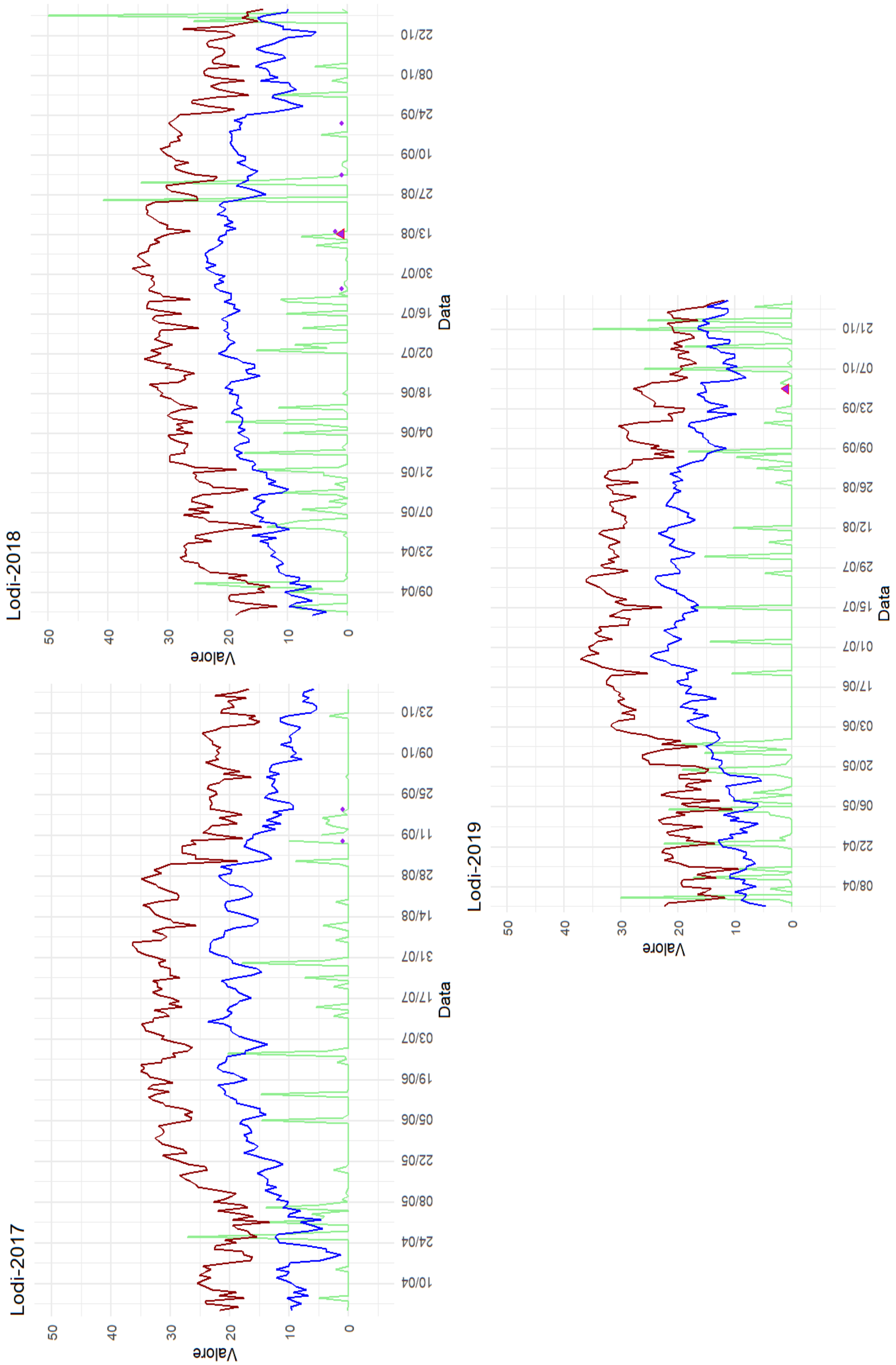

Figure 23, WNV, temperature and precipitation in Lodi, 2017-2019

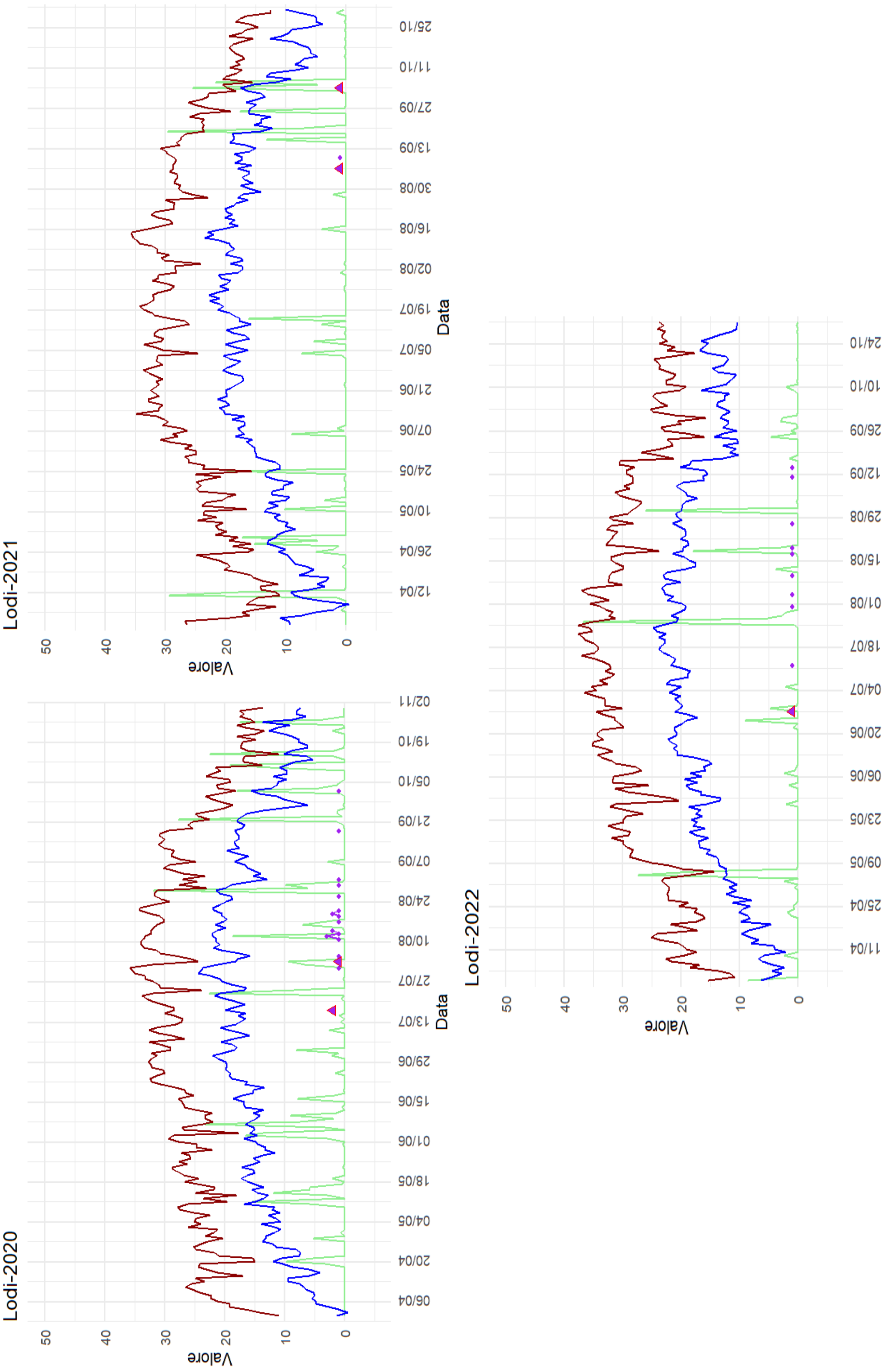

Figure 24, WNV, temperature and precipitation in Lodi, 2020-2022

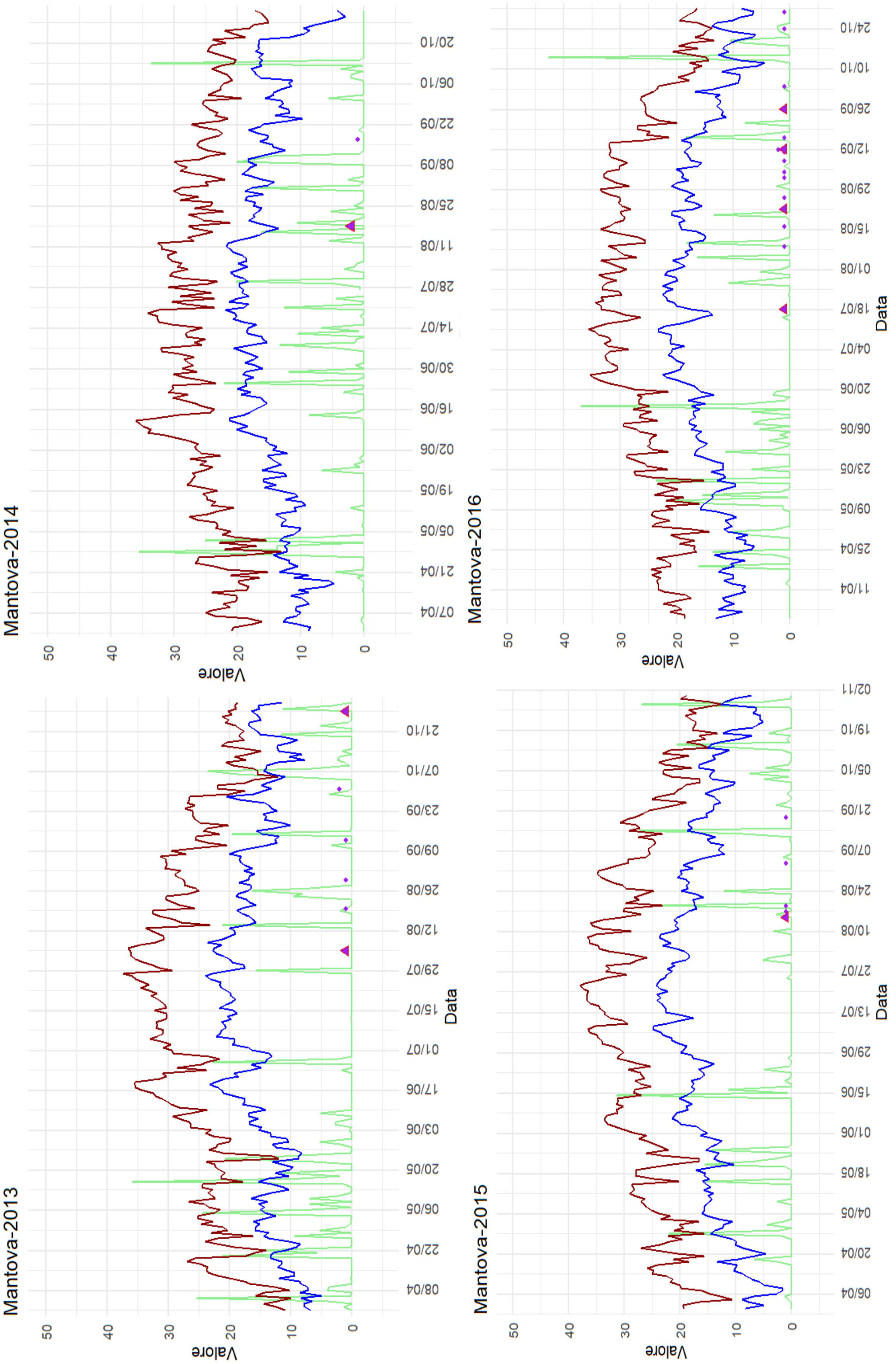

Figure 25, WNV, temperature and precipitation in Mantua, 2013-2016

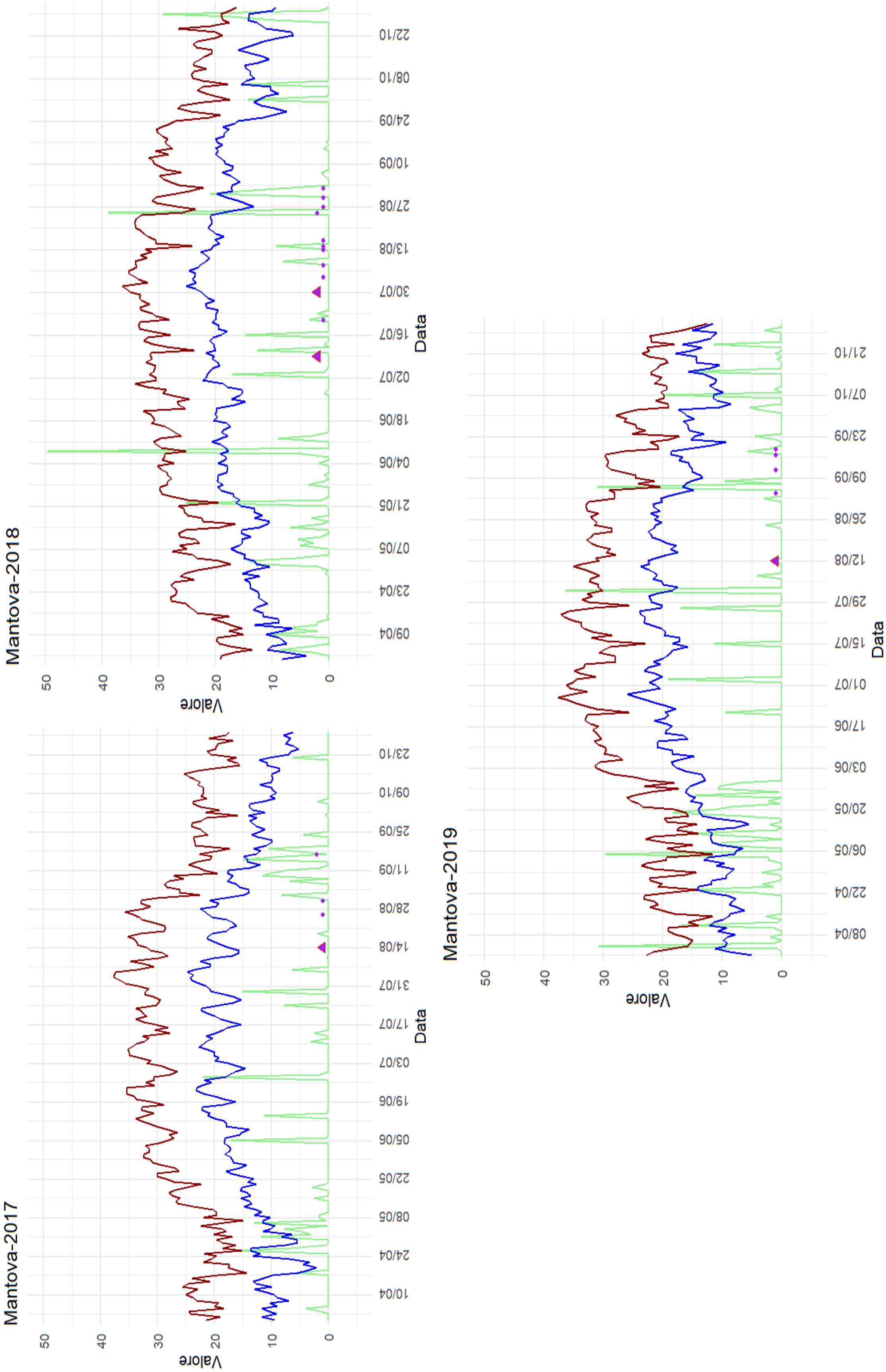

Figure 26, WNV, temperature and precipitation in Mantua, 2017-2019

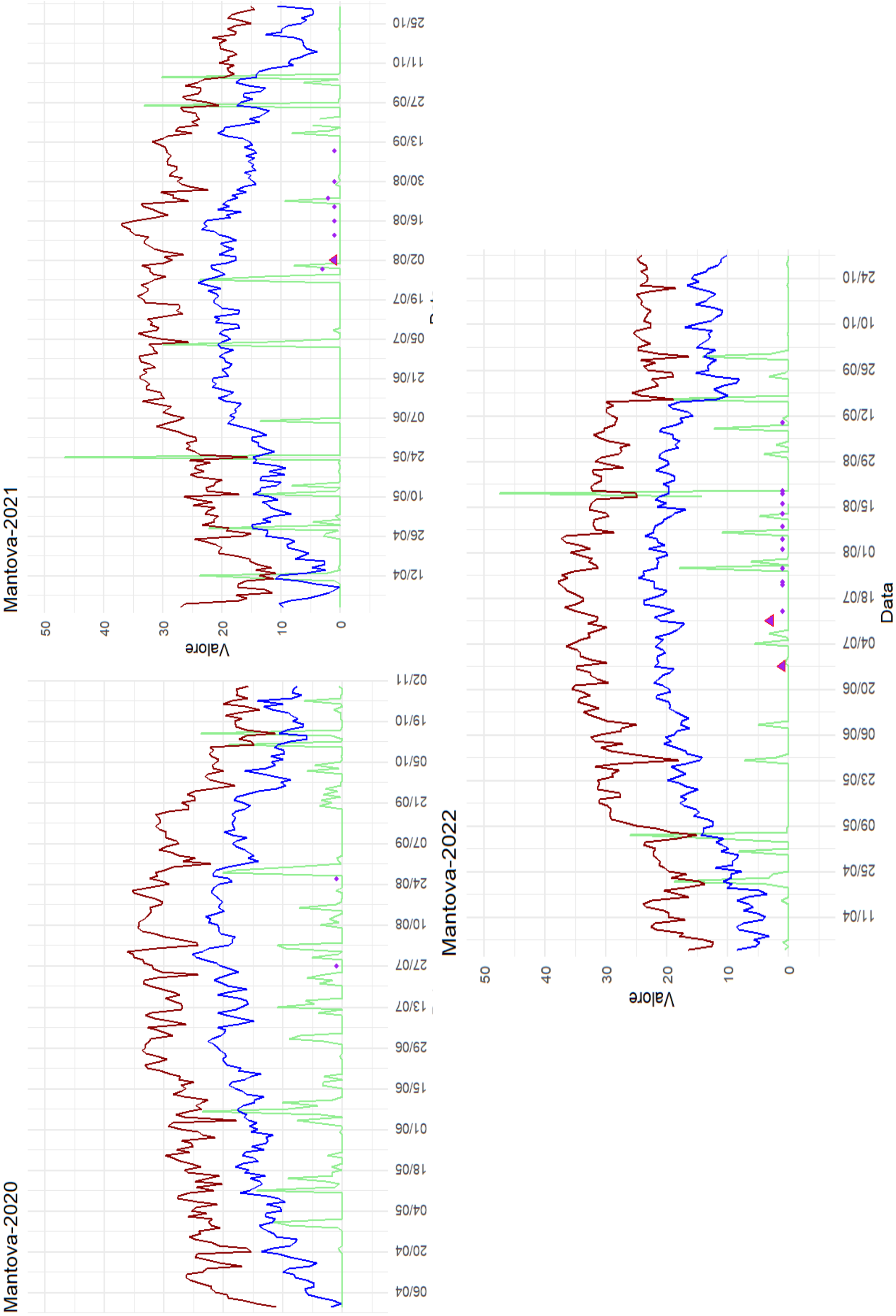

Figure 27, WNV, temperature and precipitation in Mantua, 2020-2022

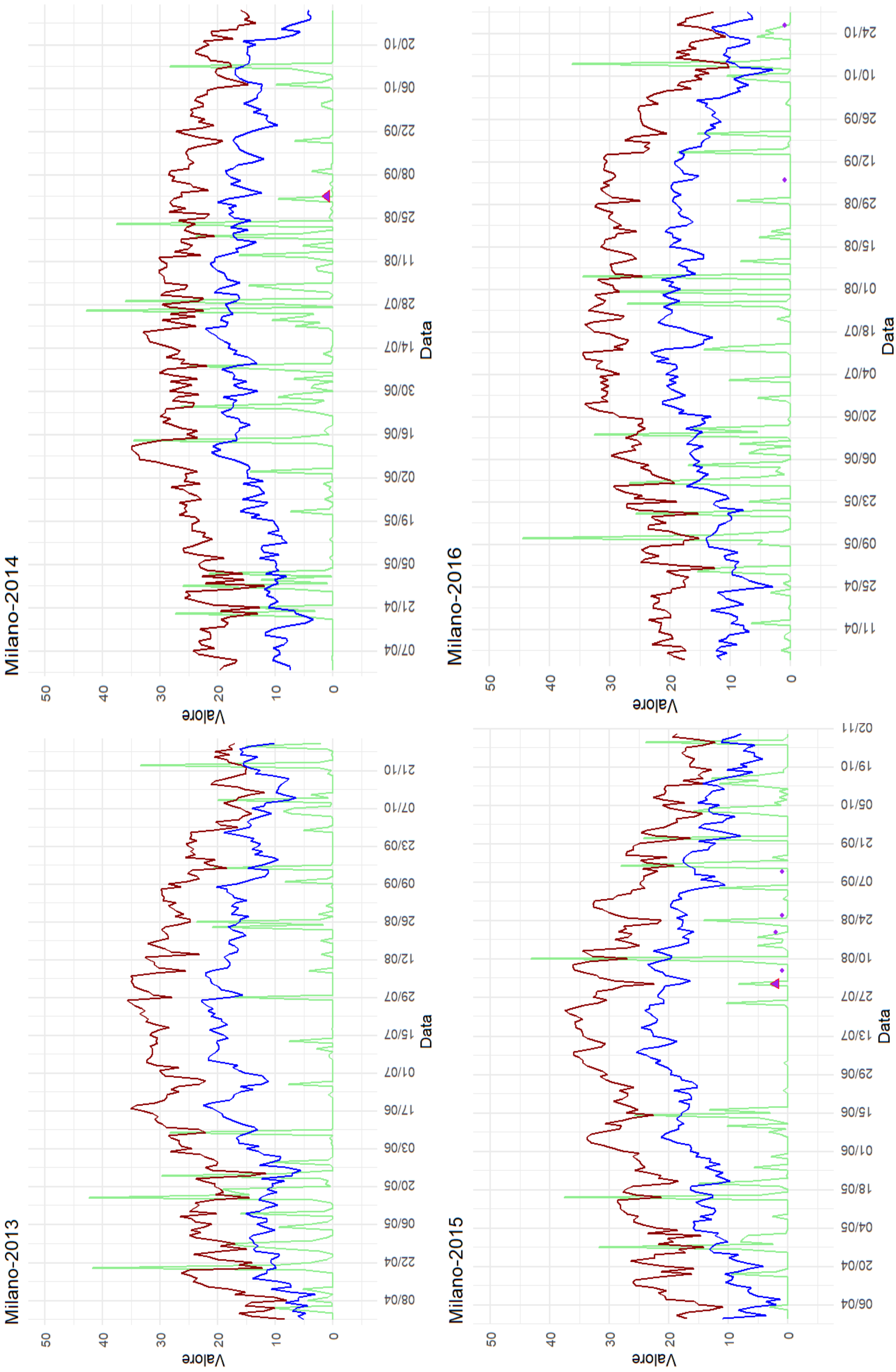

Figure 28, WNV, temperature and precipitation in Milan, 2013-2016

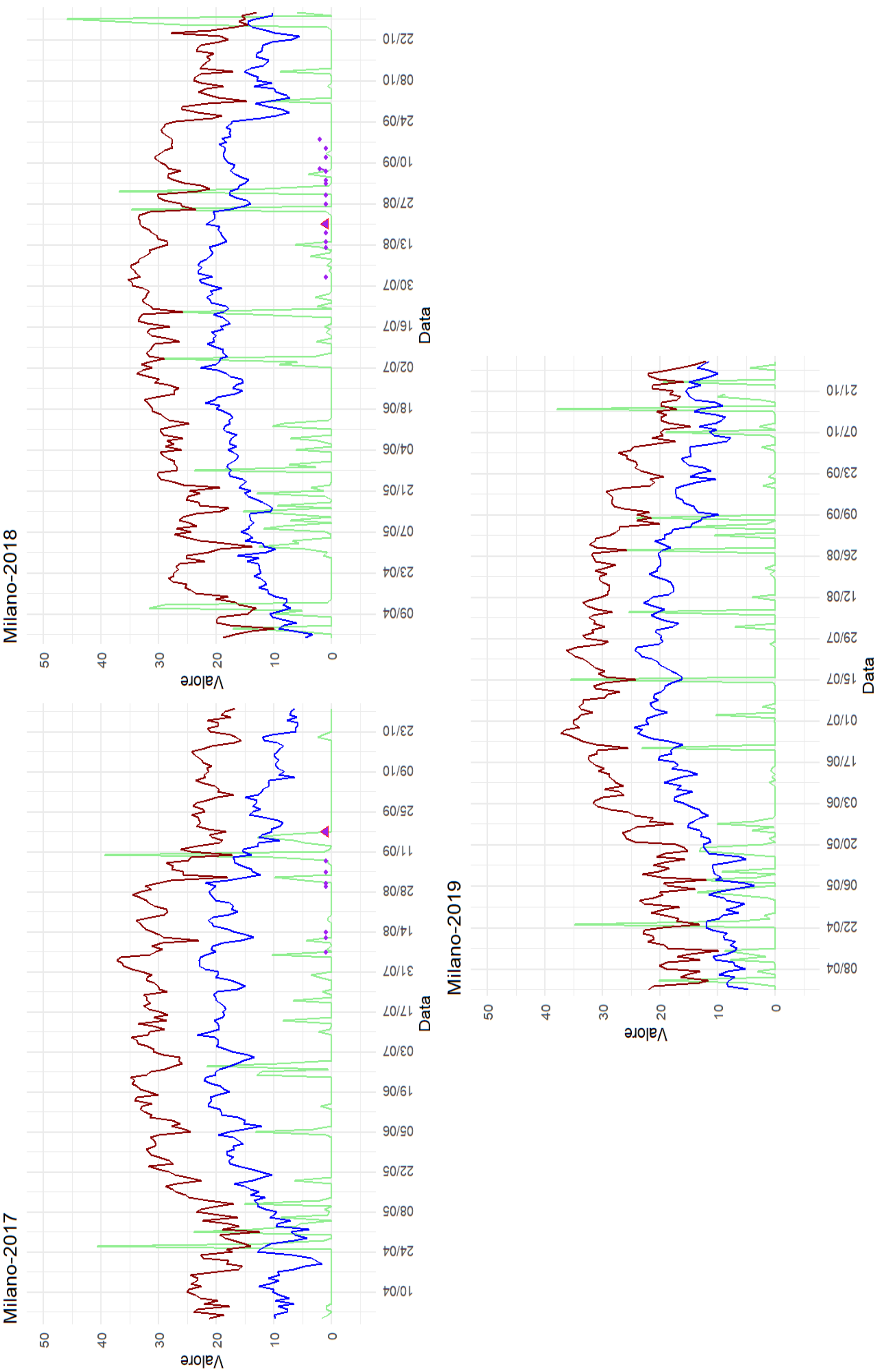

Figure 29, WNV, temperature and precipitation in Milan, 2017-2019

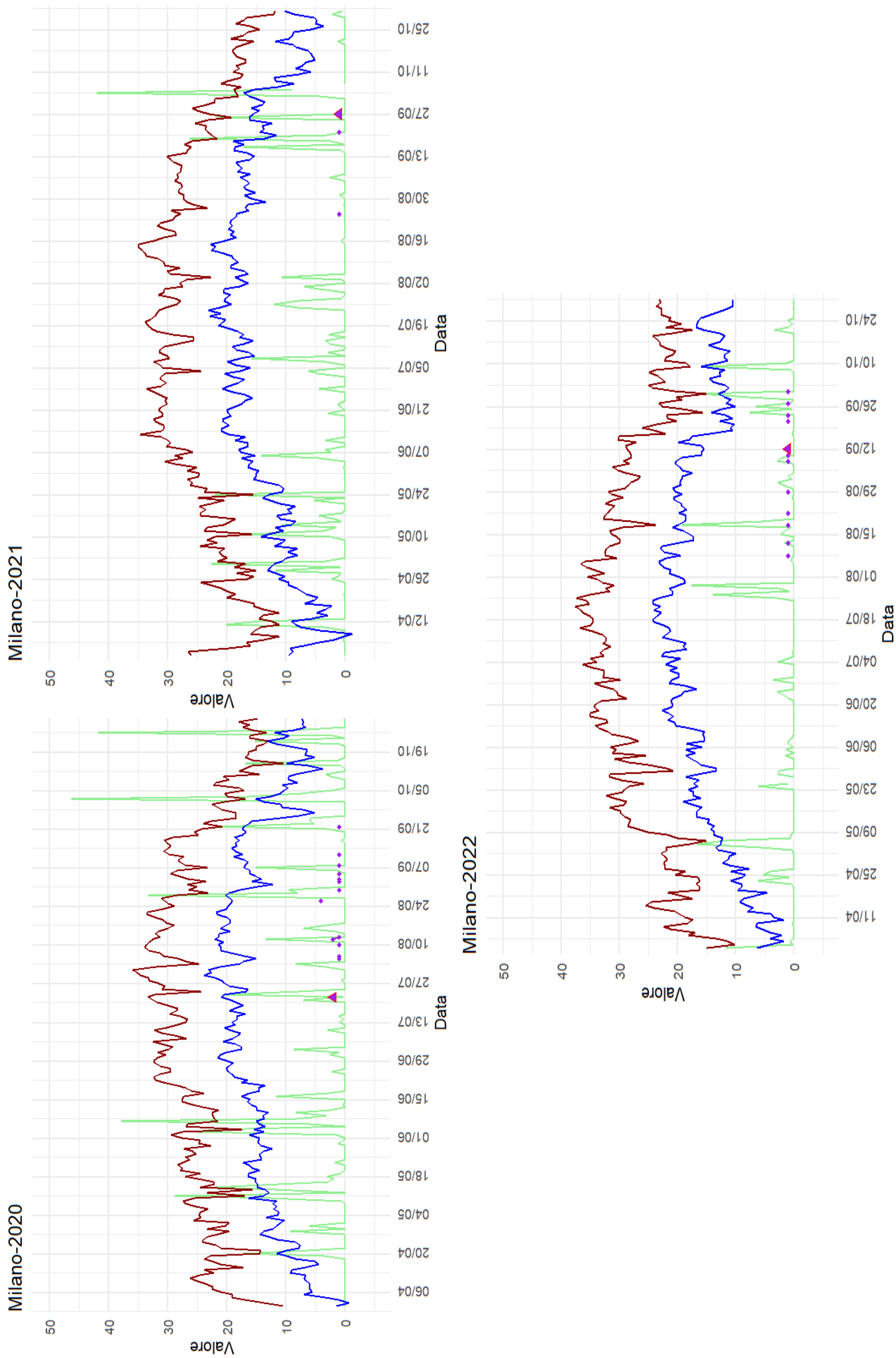

Figure 30 WNV, temperature and precipitation in Milan, 2020-2022

Prior to discussing the findings, it is important to note that a comprehensive statistical assessment on the correlation between temperature and precipitation with the West Nile Virus (WNV) is not yet available at this stage of the study and is part of a future research project. Therefore, our focus has been to produce graphical representations of the temperature and precipitation trends. The results we present in this chapter are derived mainly from visual observation of these graphs.

Upon initial analysis of the graphics, the trend in WNV cases in Lombardy has been rather uneven over the past ten years although a general increase in notifications and deaths can be observed. Exception is 2019, a year with a low number of cases.

Further examination indicates that trap positivity tends to follow thermal and precipitation peaks. Human cases typically follow trap activation, with a tendency for cases to occur after rainfall peaks but not necessarily during them.
Most traps become positive for infected mosquitos in July, while human cases emerge mainly between August and September following trap positivity.
The years with the highest case rates were 2018, 2020, and 2022. However, the effects were not evenly spread across all provinces. Generally, these years shared a climate characteristic of warmer temperatures in spring (April - May) compared to other years assessed. It is worth noting that the rainfall was unusually low at the beginning of spring and increased towards the onset of summer. Another noteworthy observation is that the peaks of cumulative rainfall correspond to deflections in both maximum and minimum temperatures.

A year of interest – that deserves further analysis – is 2019. As illustrated in *Figure 13* of the epidemiological research, only five human cases of WNV were documented that year in Lombardy. Examining 2019 climate, it is evident that summer temperatures were remarkably elevated across all provinces, whereas spring was characterized by lower temperatures and increased precipitation compared to previous years.

The following paragraphs will examine the information available by province.

Brescia

After conducting a thorough analysis of the Brescia graphs, it is evident that the province experienced a significant surge in cases exclusively in 2022, with a total of 33 cases recorded out of the 46 cases documented during the last decade. It is evident that 2022 saw markedly high temperatures in both the spring and summer seasons when compared to previous years. An additional observation highlights that 2022 was characterized by exceedingly high temperatures during both spring and summer compared to prior years. Following the initial peak of summer rain noted towards the end of July, there was a surge in human cases. This came after some mosquito traps tested positively resulting later in a considerable rise in reported cases.

Cremona

After Mantua, Cremona is the second province in terms of the number of cases, with a total of 76 cases. The cases have been distributed linearly over the years, in accordance with the region's epidemiology. It is noteworthy that there were 21 registered cases in 2022, which is consistent with the regional observations of a lower incidence in that year. In 2022 Cremona was the second province for incidence, following Brescia. The spring of that year in Cremona was marked by higher mean temperature with less cumulative precipitation.

Lodi.

In the Lodi province, cases were primarily noted during 2020 and subsequently in 2022, with significantly fewer infections in preceding years. Comparable to other provinces during this time, a spring with elevated temperatures and reduced rainfall was witnessed. In 2018, notwithstanding the higher temperatures compared to other years, the spring was characterized by a greater level of precipitation than in other provinces. This could plausibly account for the limited number of cases in 2018. In 2020, there was a notable increase in spring temperatures compared to previous years, which could account for the rise in West Nile virus (WNV) cases. Within the region, Lodi, with 27 cases out of 78 recorded, was the most affected Lombardy province in 2020.

Mantua

Throughout the years, Mantua consistently recorded the highest number of WNV infections. Graphs analysis indicates a high number of cases observed in 2016, 2018, 2021, and 2022. There were fewer occurrences in 2020, a year in which summer temperatures, whilst still elevated, did not appear to have a noteworthy effect. Nevertheless, the weather patterns in the province of Mantua during 2020 were substantially distinct from those of years with a higher number of cases. Indeed, in years with a greater number of occurrences, a time frame of several weeks without precipitation is consistently observed. In 2020, rainfall characterized the entire summer in Mantua without significant peaks and with no pause periods.

Milan

Since 2017, the province of Milan has shown an increasing trend in number of cases of WNV infection with most cases recorded in 2018, 2020 and 2022, in line with trends in the region. In those three years, temperatures were higher in the spring. In 2022, it is evident that the initial cases of WNV emerged after the re-start of rainfall in late July and early August.

Chapter IV: Discussion

WNV, a disease that currently lack a cure, is still recognized in Lombardy as an emerging zoonosis, despite its endemic status in the Po Valley for over 15 years (4). Cases reported annually in the region continue to grow and there is initial suggestion that deaths attributed to severe forms of disease may also be on the increase (6).

As discussed in detail in Chapter I, a highly advanced and sophisticated WNV surveillance system exists and has the capacity to detect infection at an early stage, enabling implementation of preventive interventions that avoid the transmission of infected blood and tissues. This excellent surveillance system also embodies the core principles of One Health, a holistic approach to healthcare premised on the fact that human health, animal health and ecosystem health are intimately interconnected. The surveillance system devised by the Istituti Zooprofilattici Sperimentali (IZS) in Italy, as described in the PNA, necessitates extensive collaboration among entomologists, veterinarians, virologists, infectious disease specialists, hygienists, public health professionals, field doctors and local authorities. It is only through the close interaction and cooperation of all these professionals that it is possible to locate, describe and respond to the WNV spread (8).

To achieve a comprehensive One Health approach for surveillance, the environmental and climatic factors that affect epidemiology annually cannot be ignored.

Thus, existing literature supports the integration of environmental and climatic components as essential for the understanding of Arboviroses (3). Vector-borne diseases thrive in environments favorable to the development of their vectors, and climatic variables play a significant role in the development of vector populations (53,76).

The study of climatic conditions is thus indispensable not only for monitoring but also for understanding the epidemiology of West Nile virus in a wider context.

Numerous studies have acknowledged the significance of climate in the transmission of the virus. However, the impact of climate and environment is extremely area-dependent and therefore it is crucial for regions with viral circulation to investigate how climate affects their specific circumstances (12).

The objective for which we carried out this investigation about how climate impacts the epidemiology of WNV in Lombardy in the 2013-2022 period is not only descriptive. We aimed to investigate the climatic factors, recognizing that due to the ongoing climate change, it is increasingly crucial to comprehend how climate affects the propagation of Arboviroses (76). This is imperative as there is a threat of the virus extending to new regions that were formerly non-endemic for WNV or had lower circulation (41). Researchers have reported a possible correlation between the epidemic WNV peaks of 2013, 2018, and 2022 in the Po Valley and the climatic anomalies that occurred during these years' spring-summer period (25,46). Studying the impact of climate on the spread of WNV in Lombardy is essential to predict the future disease burden and prepare health systems and services accordingly. Therefore, fully implementing the One Health approach, also including the climate aspect that is currently missing from the PNA, will facilitate the identification of potential risk areas and timing, thus enhancing WNV surveillance and response capacity.

Our research is the first study undertaken in Lombardy to evaluate the correlation between climate and WNV circulation. Furthermore, to the best of our knowledge, no descriptive inferential study on the epidemiology of WNV in Lombardy has ever been conducted before.

The findings outlined in Chapter 3 constitute merely the preliminary outcomes of an ongoing research endeavor, which is still in its nascent stage. As evidenced by the intricacy of the amassed data, this ambitious initiative is envisioned as an initial stride towards broad-reaching research aims. A high degree of interdisciplinary cooperation is necessary for its execution, which extends the research time.

Before examining the correlation between WNV cases and environmental factors, we extensively analyzed the available evidence on epidemiology of WNV in Lombardy during the study period. It is worth noting that the period was selected based on data availability. Specifically, 2013 marks the first year in which the Lombardy Region was able to systematically report data related to both human infections and veterinary surveillance.

We decided to consider all human cases of WNV observed in the area, regardless of classification, as the human study population. This led us to include not only confirmed cases, but also probable, possible, and unspecified cases. We also included data on diagnoses in blood donors, which can be defined as 'incidental' diagnoses, as these are asymptomatic patients whose infectious status was discovered by chance because of the donation.

We decided to keep the study population as large as possible for two principal reasons. Primarily, we are looking at the first 10 years of systematic data collection in Lombardy, which is also one of the leading regions in WNV surveillance systems. As we already mentioned in chapter 3, in the first decade of reporting the classification criteria have changed to meet the evolving needs of the surveillance system and the progress of knowledge about WNV.
An example of this is the clinical classification *(Figure 10),* which has been systematically introduced since 2019, or the presence of cases defined as "possible" in the first years of the study, a diagnostic criterion that is now outdated.

Secondly, our decision to maintain a sizable population was driven by our core research objective. Limiting our criteria would have resulted in a loss of crucial information about the scale of the issue and the geographic distribution of WNV infection. Eliminating probable, possible, or unclassified cases would have resulted in a loss of approximately 28,5% of the data, as shown in *Figure 11*. This is a significant and evident reduction, when considering the total of 428 cases recorded during the period, particularly since WN infection is often largely underdiagnosed. One should notice that the majority of WNV cases have an asymptomatic course; hence, they do not require medical attention, thus escaping clinical diagnosis, and, consequently, causing several difficulties in tracing the actual epidemiology of the virus. This aspect, supported by the literature, is also confirmed in our analyses. Subtracting nonclinically classified cases – as we showed in Figure 11 – one can see that in Lombardy, diagnoses of infection were predominantly incidental, coming mainly from donor patients (37%), followed by neurological (WNND - 35%) and febrile cases (WNF – 22%).

Our epidemiological analyses revealed a significant higher incidence among males, an observation also made in other relevant studies. Additional research is required to identify the underlying cause of this phenomenon. It is important to note that some literature acknowledges that external occupational exposure, which is higher in males, does not adequately explain this finding (4).

Our research corroborated a higher occurrence of cases in the Po Valley region, specifically in the Lombardy southernmost areas of Cremona, Mantua, Milan, Lodi, Pavia, and Brescia *(Figure 9).* Furthermore, an initial assessment of case frequency annually revealed that Lombardy experienced a surge in WNV cases during 2018, 2020 and 2022 *(Figure 13)*. This trend is also evident on a national scale: these are the years during which Italy experienced a higher number of reported cases. However, the peak in Lombardy in 2020 was more significant than in other regions. In Lombardy, 2020 has the second highest prevalence rate after 2022, while in Italy it is the third highest after 2022 and 2018 (32).

As this is a medical thesis, our primary focus is clinical. In the context of this One Health project, our primary concern is the impact of climate on human patients.
To achieve this, we initially concentrated our analysis on the five WNV most affected provinces in Lombardy: Cremona, Mantua, Milan, Lodi, and Brescia. These are provinces situated in the Po Valley that have experienced the effects of WNV over the years, with notable fluctuations between different years.

The initial findings, while rooted in the inferences gleaned from the graphs, enable us to draw some conclusions.

As the existing literature presumes about the climate, observing the connection between temperature and rainfall during the spring season, we note a comparable pattern in the most impacted regions of Lombardy during peak viral circulation periods (2018, 2020, and 2022). During April and May, temperatures tend to be higher than in previous years, with an increase of up to +5°C. Rainfall sees its peak in June, which is close to the beginning of summer. As discussed in Chapter 1, this climatic pattern is favorable for the concentration of vectors and reservoirs in wetlands, leading to the creation of ecological niches. Consequently, this favors the interaction between mosquitoes and birds, resulting in viral amplification (13).

This correspondence appears to be due to the fact that a year such as 2019, which was marked by an exceptionally hot summer following a spring season that experienced lower temperatures than other years and was much wetter, had minimal viral transmission (only 5 cases were reported in the entire Lombardy region during that year).

The temperature fluctuations observed align with the consequences of climate change. Years characterized by temperature swings and reduced rainfall are the direct result of the climatic changes occurring in our territory. Such events have been observed in close proximity in recent years (59,60).
The rise in temperature appears to promote the adaptation of novel vectors, once restricted to tropical climates only, such as the Aedes koreicus and japonicus mosquito species. These new vectors facilitate the transmission of arboviruses into the region. If this temperature trend continues, it may lead to a more significant surge in cases of WNV and other arboviruses (76,77).

It is noteworthy that the incidence of human infections reaches its peak during the August-September timeframe, which corresponds with the mosquito's altered feeding habits due to a decrease in bird availability (16).

Upon an initial analysis of the graphs, it appears that the correlation with temperature is more apparent than that with rainfall, aligning with findings from studies conducted

in nearby regions (39,53,78). The peaks of positivity in traps and human cases consistently trail thermal peaks. Although there is no direct match, this could be explained by a latency window between infection and positivity, due to the viral incubation period (27,51). However, rainfall also holds significance. It is noteworthy that mosquito positivity does not decrease during rainfall, as heavy rains can harm mosquito habitats. Nevertheless, rains enhance the mosquito larval cycle, and the consequent positivity of traps supports this observation (26).
Assessing rainfall can be challenging because peak rainfall often coincides with a drop in temperature. Rain is essential for mosquitos, as previously mentioned, but higher temperatures also have beneficial effects on the mosquito's life cycle, which accelerates (47).

We aim to conduct robust analyses in the future, enabling precise discussion of thermal correlation in relation to temperature degrees and precipitation. We will provide statistical value to our observations and quantify the time between climatic peaks and positivity. This data is essential for future developments in WNV surveillance.

This study represents an innovative breakthrough for the Lombardy region. As stated throughout this dissertation, objectively evaluating the relationship between climate and WNV infections proves challenging due to its heavily area-dependent nature, influenced by local conditions, which hinders cross-regional comparisons. Nevertheless, our research findings can be compared to those of neighboring regions such as Piemonte (53), Emilia-Romagna (39) and Veneto and Friuli Venezia Giulia (28,78).

In these regions as well, it was hypothesized that the warmer and less rainy spring could potentially have a significant impact on viral epidemiology. It should be noted that the studies conducted in these areas differ from ours and are complex to compare methodologically, even among themselves. Nevertheless, previous research indicates that spring temperature plays a significant role in determining the subsequent summer viral spread. The key impact of temperature is the acceleration of the virus extrinsic incubation period in the mosquito. Fornasiero and colleagues(28) have created a model to research the diffusion of WNV in the area by assessing a greater number of variables than our study and emphasizing the impact that variables have on mosquitoes, rather than on spread. The study's assessments were complex, but the results aligned with those of previous studies. The interaction between variables is area-dependent and intricate, and it is crucial to monitor the thermal trend of the spring season. This pattern appears to have predictive significance regarding the subsequent mosquito epidemiology.

We have seized on this discourse to analyze the principal practical implication of a study that establishes a correlation between climate and WNV. This climate data has the potential to revolutionize Italy's Surveillance System due to its absence of climatic parameters. Currently, the Italian peninsula is classified according to infectious risk in the PNA, focusing on the environmental aspect as a key factor in viral spread. Indeed, areas are classified as high risk based on the circulation of WNV in the territory within the previous 5 years, and also based on eco-climatic compatibility. Unsuitable ecoclimatic conditions are found in low-risk territories and favorable ones in intermediate-risk areas, however, without further explanations (8).

However, there is a lack of real integration of the climate aspect in risk assessment when understood in a dynamic sense. Monitoring of traps and birds is carried out to assess whether the virus is circulating, but climate monitoring upstream of these actions is absent despite literature recognizing a role for climate in dictating viral epidemiology.

Accurately describing this climate correlation would enable integration of relevant information into the surveillance system. This could be achieved by incorporating annual epidemiology development with climate analysis for the at-risk areas. By doing so, the One Health value of our surveillance system could be further enhanced. Neglecting the climate aspect at present suggests that WNV surveillance in Lombardy and Italy is a static model. Sampling commences on June 1st every year as it marks the onset of the case season, typically during the late spring-early summer period. However, the first trap positivity displays significant inter-year variation upon

analyzing the graphs, occurring at times either before or after June 1st. Including climate information in the model could change it from static to dynamic, altering the timing of trap assessments to match the requirements dictated by the seasonal climatic characteristics.
This could also result in potential cost savings. Furthermore, it would add dynamism to the surveillance model by enabling the identification and prediction of those areas, previously deemed to have a medium or low risk, which may experience a significant increase in risk on an annual basis.

It is essential to acknowledge the limitations of this study.

The study has an inherent limitation, namely the limited data and the small-time window. Conducting statistically significant studies with only 428 cases over a short time span is challenging. We acknowledge that WNV cases exceed those that are identified through diagnosis, resulting in underreported notifications. Although the low incidence of the disease is favorable, researching this disease's obscured iceberg proves challenging.

Furthermore, our initial Lombardy-based epidemiological study encountered challenges when interpreting data related to human WNV cases due to objective difficulties. Although WNV case histories are retrieved yearly with punctuality, critical sections of the forms are often incomplete leading to profound information loss. An illustration of this is the summary of consequences and follow up. Among the 428 human cases, 121 results are indeterminate, 75 non-relevant (relating to donors), 73 progressing, 109 healed, 17 serious, and 33 deceased. As inferred from the chart, in most incidents of tracking improving or severe patients, no progress records exist. With an excess of unidentifiable cases (28.3%) and inadequate data about monitoring, any statistical analysis over the whole timeline is arduous. To investigate this aspect in the future with more rigor and evidence, a study would need to gather the missing information on outcomes from patients' medical records, subject to ethical approval.

Additionally, clinical classification, which was previously based solely on laboratory criteria, has been systematically introduced since 2019. This has complicated our ability to estimate how many cases of WNND really were during the study period, which is a remarkably interesting parameter to know because, as we have already pointed out in the discussion, WNND cases constitute the true tip of the invisible iceberg of WNV infection. This implies that, knowing WNND number cases and considering that they are only 1% of infections, one can estimate the real viral circulation in a territory. A potential area of interest for future epidemiological research would be to reclassify cases based on the symptoms exhibited by patients infected with WNV prior to 2019.

Our study covered the period from 2019 to 2022 (see Figure 13), during which cases and outcomes were reported with greater precision. However, the data are not sufficient for meaningful statistical analysis.

Moreover, reporting of positivity dates for diagnostic tests was sometimes lacking, and the time interval between testing and notification to the ISS varied, potentially resulting in errors in the correlation between climate and human health, and a loss of timely information.

Turning our attention now to the climate observations.
The outcomes from these findings provide valuable guidance for our research. Nonetheless, further scrutiny is required to precisely determine how climatic factors affect epidemiology. While observing a correlation between temperature, rainfall, and viral circulation, we are yet to quantify their impact accurately.

Another restriction of our ecological research is that the interplay between climate, environment, and Arboviroses is exceedingly intricate. While temperature and precipitation have been identified as the primary climatic drivers of WNV epidemiology in the literature, they are not the only climatic factors involved. It would be valuable to conduct a comprehensive study and develop a model to evaluate the impact of other climatic variables, including relative humidity, drought, and the effects of extreme weather events, which are expected to increase in the future according to climate predictions. This study did not assess the influence of environmental factors such as irrigation and human activity in the Po Valley region, as previously highlighted in the introduction to this thesis, which can affect the vectors and subsequently impact the virus's epidemiology. It may be worthwhile to quantify the effect of these variables on WNV's epidemiology in a future predictive model and incorporate it into the research.

One aspect not covered in this study, due to a dearth of available veterinary information and is considered to require future investigations as per the opinion of veterinarians involved in this study, is the intricate role of birds, which act as reservoirs and amplifiers of the virus.
The introductory chapter indicates that migratory birds have a crucial role in introducing the virus to fresh areas, while the development of infection among local birds leads to the virus being prevalent. Currently, the duration for which a species can maintain a viraemia and its potential for developing immunity to the infection is unknown. In addition, it is unclear whether the virus continues to circulate within an area if reservoirs are immune due to avian immunity in local species.

Continuing on the veterinary limits, it is necessary to devote a paragraph to the entomological aspect. The mosquito-vector can be considered the most intriguing aspect to examine from a One Health standpoint since they represent the connection between climate change and human cases. Increased temperatures and changes in rainfall benefit the vectors, resulting in their increased survival within a bird reservoir environment, which in turn leads to an increased spread of viruses and potential transmission to humans.
The issue at hand is the paucity of information on mosquitoes that can be procured from the One Health surveillance system at present. During the surveillance period, mosquito traps undergo bi-monthly checks to detect the first instance of positivity. No other information is collected. Once WNV is detected, the trap is deactivated, and the area is designated as an infectious risk zone. Although this information is adequate for surveillance, which strives to test donors, avoiding infected transfusions and

transplants, it falls short of providing a comprehensive One Health perspective. The absence of data on the infection rate of collected mosquitoes, or insufficiently frequent surveys on traps, hinders a meaningful evaluation of the direct connection between vector and climatic variables, a relationship already known to exist. Consequently, obtaining data on the actual effect of the environment on the mosquitoes and their infection with WNV would provide greater robustness to any future predictive climate models.
Studies to acquire more precise information regarding mosquitoes are presently in progress in certain regions of Lombardy by University research groups, but as far as we know there are no comprehensive studies planned by IZSLER for the whole of Lombardy.

Concluding the analysis regarding the constraints identified in this investigation, it should be noted that, as previously highlighted, this research is the initial one of its kind in Lombardy. Although the findings are intriguing, it implies that there are no real comparisons for our results with the literature, when we consider this geographical area. It also should be noted that climate change in the Po Valley has led to catastrophic events, including floods and heat waves. While these events could harm mosquito development and thus potentially impact the epidemiology of WNV, it is important to recognize their substantial impact on human health. These situations should be considered when preparing the healthcare system for climate change emergencies (59,60).

From the discussion of the limitations, several insights for future scientific research emerge. In order to fully understand the impact of WNV epidemiology in Lombardy, a more detailed epidemiological analysis needs to be carried out, reclassifying cases by clinical features before 2019. It is important to trace back the cases of WNND to accurately estimate the actual spread of the virus in the Lombardy region during the study period. In this context, it is also crucial to recognize the importance of a more accurate tracing of deaths due to WNV. For the future, it is important that notifications to the ISS are more complete and that the dates of positive reports are accurately recorded: having the precise dates of diagnosis, in addition to the dates of reporting to the platform, makes it possible to increase the solidity of the relationship between climate peak and increase in human diagnoses. It is also important to understand why the male population is more affected than females.

One question that remained open in our comparison with the Lombardy region was: how many WNF arrive at the emergency department in summer that could be misdiagnosed as fever of unknown origin (FUO)? Studying this aspect could be particularly important to quantify the real impact that WNV infection has had on the health system in recent years, in order to assess whether it is necessary to increase the resources devoted to WNV by the regional and national health systems. Quantifying the economic impact may indeed dictate the allocation of resources, including for research into surveillance, treatment, and prevention (such as research into a vaccine).

It has been mentioned many times, but it is worth reiterating at this stage that these results are only the beginning of the research project on the relationship between climate change and WNV in Lombardy. Further analysis is needed to assess more precisely the impact of climate change and its quantitative impact on the increase in infections. This step is essential to integrate climate change into WNV surveillance in a meaningful and effective way. At present, we can only observe the trend.

In the future, it may be worthwhile investigating additional climate parameters, such as RH or drought indices, for which there is still a lack of evidence in the literature. The impact of climate on both migratory and resident birds as well as mosquitoes could also be assessed to develop a comprehensive predictive model, providing valuable insights for the health system in preparing for future challenges.

Further research is required to determine whether there was a climatic correlation in the past 15 years which facilitated the endemic spread of WNV in Italy. If this hypothesis is confirmed, it could enable the prediction of future WNV risk areas with global implications. All of this would strengthen the current evidence of a correlation between WNV, or more broadly, Arboviroses, and climate change, providing additional solidity to the presence of the health impact of climate change.

We conclude this discussion by reiterating the importance that any research effort on WNV requires a One Health collaboration of several aspects. Only multidisciplinary but coordinated research can lead to significant results: an example of this is the fact that it is essential to implement entomological information in order to have a comprehensive quantification of how climate affects mosquitoes, and consequently WNV, and humans. Our approach only led to the results we observed because we answered the research questions by consulting experts in the various fields, who were able to guide our work significantly due to their specific expertise.

Conclusions

This study offers valuable insights into the association between the West Nile virus and climate change. The findings suggest that a climatic correlation is plausible, emphasizing the need for further investigation to comprehend this intricate relationship fully. The connection between the escalation of global temperatures, alterations in rainfall patterns and the prevalence of WNV creates fresh possibilities for comprehending the propagation and epidemiology of this ailment.

It is imperative to maintain our commitment to continuing this research, always with a One Health approach. Additional studies have the potential to enhance our scientific comprehension WNV infection and lead to the creation of more efficient prevention and control approaches, resulting in genuine advancements in public health.

Climate change is a current challenge, and it will progressively become a more serious challenge in the near future. Several studies and predictive models describe future climatological scenarios, depending on whether measures are implemented to alleviate the climate impact. It is indisputable that over the next 50 years, the climate in the Po Valley and Lombardy will experience major changes that will result in consequential impacts on public health. Therefore, adequate preparation is required. Addressing climate change presents an unparalleled prospect for global health advancements in the 21st century. As healthcare professionals, we bear a civic duty to raise awareness among politicians to take action concerning the development of climate adaptation policies before it is too late. Comparable to the Covid 19 pandemic, it is necessary to make decisive choices to address these issues. The impact of climate change on health is already tangible, as demonstrated by this study. As demonstrated by the implementation of the One Health approach to arbovirus analysis, we have the capacity to understand the current situation and anticipate future events. We possess the expertise for this, now action is needed.

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