Quantitative analyses of human West Nile Virus outbreaks in Greece, Hungary, Italy and Romania, 2010-2015

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Declaration of Authorship

I, Aphrodite Spanou, hereby declare that the work contained in this thesis and the work presented in it is entirely my own and I have clearly documented all sources and materials used. I received supervisory advice and comments from Prof. Tony Gatrell, Prof. Ceu Mateus and Dr Luigi Sedda, during the writing up of this thesis.

This work has not been submitted in any form for the award of a degree at this university or any other institution, nor has this work been published.

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ABSTRACT

West Nile Virus (WNV) is a non-communicable, geographically sparce, mosquito borne disease with considerable consequences for human health both at individual and collective level. Since its first isolation in 1937, several geographically spread sporadic, endemic and epidemic outbreaks of WNV disease have been recorded in every continent, except Antarctica. WNV is now the third most prevalent zoonotic febrile infectious disease in the EU. There is a gap in the literature that of synthesising multifactorial data relevant to successive human outbreaks in Europe. To address this gap, this study explored epidemiological, hydro-climatological and environmental data to make meaningful inferences about the composition of human epidemic outbreaks in Europe between 2010-2015. The analysis was based on secondary data collected from the ECDC (European Centre for Disease Prevention and Control), EUROSTAT and ECMWF (European Centre for Medium-Range Weather Forecasts).

ECDC and EUROSTAT data were employed to generate the epidemiological profiles of human WNV outbreaks in Hungary, Italy, Greece and Romania which experienced consecutive outbreaks during the studied period. It showed that the ratio of men contracting WNV infections compared to women was higher across all years and countries (Hungary 1.7 (95% CI:1.1 to 2.5), Greece 4 (95% CI:3.4 to 4.8), Italy 4.7 (95% CI: 3.7 to 5.9) and Romania 1.7 (95% CI:1.29 to 2.4)). WNV occurred mostly in those over 65 years of age apart from Hungary (0.3 CI: 0.2 to 0.5) which reported most of its cases in those between 45-64 years of age. It also explored environmental and landscape factors influencing WNV's distribution across the four south eastern and southern European countries experiencing recurring epidemics. The results suggest that human West Nile disease transmission between 2010 and 2015 occurred in both coastal and non-coastal areas but 63.7% of cases, across the four countries, were detected in non-coastal areas. This result may have been skewed as Hungary's geography is land locked and Greece did not report any cases in 2015. The study also explored urban, intermediate and rural areas in relation to human WNV outbreaks and the analysis showed a mixed picture. The rise of infections in intermediate and urban areas may indicate that residential infrastructure or population concentration may generate more larval growth which may have led to the outbreaks, but this assertion requires further investigation.

Hydroclimatic data on air temperature, dewpoint temperature, soil temperature, total precipitation, relative humidity, surface pressure, volumetric soil water, wind components U (east facing wind velocity) and V (north facing wind velocity) were extracted from the ECMWF's Re-Analysis (ERA-Interim) system. A zero inflated mixture framework was employed as a means of assessing the relationship between these hydroclimatic parameters and human WNV incidence for the same countries and years. Three nested models were tested: lag0 (same month as the outbreak), lag1 (a month before the outbreak) and lag2 (2 months before a human WNV outbreak occurred). lag2 was the best fitting model. Apart from soil temperature and the V wind component, all other parameters were significant and highly associated with human WNV disease outbreaks in the best fitting model. Further research is needed on subsequent years to confirm the robustness of these findings.

Keywords: West Nile virus, epidemiology, landscape typologies, hydroclimatic parameters, zero-inflated models.

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CHAPTER 1: INTRODUCTION

1.1 Problem Statement

West Nile Virus (WNV) is an emerging, mosquito-transmitted, viral agent which, together with other important human and animal pathogens, belongs to the Japanese encephalitis virus (JEV) antigen complex serogroup (Pauli et al., 2013; Mukhopadhyay, Kuhn and Rossmann, 2005). It was first detected in 1937 in Uganda, near its north-western West Nile periphery, from blood sampling of a human case demonstrating pyretic (relating to, or characterised by fever) symptoms (Smithburn, Hughes, Burke and Paul, 1940). It is estimated (ECDC, 2018; World Health Organization, 2018; Sambri et al., 2013; Davis, 2004) that:

- the vast majority of human infections are asymptomatic (80%),
- around 20% cause West Nile Fever (WNF) and,
- around 1 in 150 persons (less than 1%) develop more severe neurological complications, West Nile Neurological Disease (WNND), such as encephalitis or meningitis or WN poliomyelitis. Other neuropathies, including the Guillain–Barré syndrome, have also been detected (Petersen, Brault and Nasci, 2013) but are less frequent.

The virus has been found in forty-three mosquito species, primarily of the genus *Culex* (Kulasekera et al., 2001; Hurlbut, 1956) which are regarded as the most prominent maintenance vectors for WNV. *Culex* mosquitoes and birds' interactions tend to intensify near waterholes and/or standing waters during periods of drought (Patz, 2003), especially in deltaic and other wetland ecosystems. This suggests that certain environmental typologies play or contribute to WNV occurrence in humans and equids. Birds have also been proven to be an attractive reservoir host to Culex spp. (Gangoso

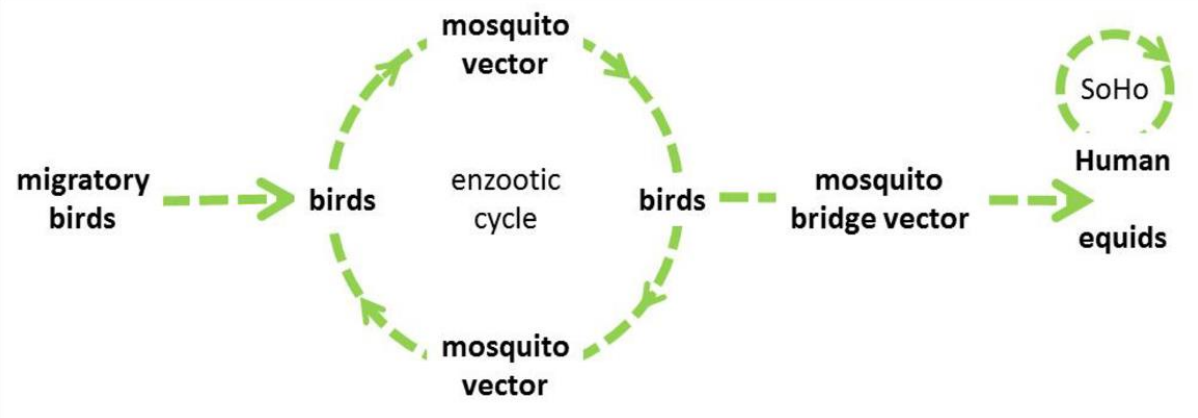
et al., 2020; McIver, 1968; Allan, Bernier and Kline, 2006). The latter become infected during blood meals on viraemic avian species, capable of infecting more birds and other vertebrates during consequent feedings (Pauli et al., 2013). Birds become amplifying hosts when infected (Komar, 2003) whereas humans and other mammals, infected from a bite of a viraemic mosquito, are generally considered accidental dead-end hosts (ECDC, 2018; Campbell, Marfin and Lanciotti, 2002).

Mosquitoes become infected when they feed on infected birds and “after the virus enters through the gut wall into the haemolymph, it replicates in most of the internal tissues and eventually arrives in the mosquito's salivary glands” (ECDC, 2013, p3). Once infected, adult Culex female[1] mosquitoes remain infectious throughout their lifespan which is estimated to be around 2-4 weeks, subject to climatic and a variety of other factors. Mosquitoes’ full life cycle can range from just four days or up to one month, subject to hospitable weather conditions. Specifically, mosquitoes tend to be more active in hot temperatures at 27 °C, dormant at 16 °C and unable to function at 10 °C or under (Rutgers, 2001). Soverow et al. (2009) found positive human WNV associations in 17 states in the USA with increasing temperature four weeks prior to symptom onset. Anderson et al. (2008), Richards et al. (2007) and Dohm et al. (2002) found that the incubation period[2] lasts 10 to 14 days during warm weather conditions which suggests that climatic conditions, close to or preceding the occurrence of an infection, may contribute to WNV circulation. Further, increased rates in bites indicate the presence of a breeding site especially since mosquitoes do not tend to move far from their larval habitat or fly more than 3km (CDC, 2014).

[1] Culex males feed on flower nectar. Only female require blood meals.
[2] The incubation period is the number of days between when you are infected with a disease and when you might develop symptoms

The relationship (Table 1) between feeding patterns of *Culex spp*. On birds (enzootic transmission) and then humans and animals (epizootic transmission) infections are relatively well evidenced in published literature (Kilpatrick et al., 2006).

Table 1. West Nile virus transmission cycle



Source: ECDC, 2018, page 3. SoHo: substances of human origin

Since its first isolation, several geographically spread sporadic, endemic and epidemic outbreaks of WNV disease have been recorded in every continent, except Antarctica, and mainly, but not exclusively, in temperate environments marked by either moderately high or high temperatures (Gubler, 2007). Following the severe outbreaks in Romania's capital city, Bucharest, in 1996 (393 hospitalised cases & 17 deaths) (Hubalek and Halouzka, 1999), several EU and neighbouring countries[3] have been reporting locally acquired human WNV cases (Haussig et al., 2018) and a substantial increase in West Nile Virus infections in human populations (ECDC, 2020). The above highlights a noteworthy trend of the epidemiological situation of WNV in the European Union (EU) and its neighbouring countries.

[3] Austria, Bulgaria, Croatia, Cyprus, France, Greece, Hungary, Italy, Kosovo, Portugal, Romania, Spain, Serbia and Turkey

Research evidence suggests that WNV is now endemic and possibly permanently located in many European regions (Koch et al., 2021; Danis et al., 2011; Papa et al., 2010; Krisztalovics et al., 2008). Young et al. (2019) and previously Calistri (2010) argued that an analysis of WNV infections and outbreaks in Europe is now more possible and necessary. However, the use of consistent European level data sources on the epidemiological and spatiotemporal patterns as well as hydroclimatic[4] and environmental factors surrounding the consecutive occurrence of human WNV outbreaks in EU countries has not been fully explored.

Over the last decade (between 2009 and 2018), a number of EU/EEA and EU enlargement countries (Austria, Bulgaria, Croatia, Cyprus, France, Greece, Hungary, Italy, Portugal, Romania, Spain, Serbia and Turkey) reported 3,362 locally-acquired (sporadic as well as large outbreaks) In more recent years (2018); this presents a dramatic 7-fold increase (n=1605) in WNV disease circulation in the EU compared to previous years, making it the third most prevalent zoonotic febrile infectious disease in the EU after tick-borne encephalitis (TBE) (n=3,212) and dengue virus (DV) (n=2,191) (ECDC, 2018). EU Member States reported 316 locally acquired human cases of WNV infection in 2020, down by 94 compared to 2019, with a number of regions in Bulgaria, Spain, the Netherlands (Vlaskamp et al., 2020) and five regions in Germany recording autochthonous human cases of WNV infection for the first time (ECDC, 2021).

The burden of disease caused by co-circulation of and co-infection from multiple infectious diseases has been explored for some zoonotic infections (Carrillo-

[4] Hydroclimatology was defined by Langbein (1967) as the "study of the influence of climate upon the waters of the land." It includes hydrometeorology as well as the surface and near surface water processes of evaporation. **Source**: Wendland W.M. (1987) Hydroclimatology. In: Climatology. Encyclopaedia of Earth Science. Springer, Boston, MA. https://doi.org/10.1007/0-387-30749-4_90

Hernández et al., 2018) including WNV (Zannoli and Sambri, 2019). Flaviviruses and alphaviruses are by far the most commonly occurring arboviruses worldwide (see Annex A). Tick-borne encephalitis (TBE) virus, a flavivirus, records 5000-12000 human infections in Europe each year (ECDC, 2014). Phleboviruses, such as the Rift Valley fever closely related to West Nile Disease, have the potential to enter Europe mainly through transportation of animals whereas viruses belonging to the Reoviridae family such as bluetongue virus (a livestock disease) are already in circulation in Europe (Gould, 2006). In addition to WNV, Usutu virus (Clé et al., 2019; Vilibic-Cavlek et al., 2020), Sindbis virus and Tahyna virus, have also been detected in birds even in colder European climates such as that of the UK (Buckley et al., 2003). This signals that Europe's environment is attracting evolving neurotropic diseases.

The emergence of other Mosquito-borne flaviviruses (MBFVs) (Annex A) such as Chikungunya (CHIKV) and Rabensburg virus, may create additional pressures for European authorities managing the spread and transmission of multiple seasonal non-communicable infectious diseases (Beltrame et al., 2007; Bakonyi et al., 2006) at the same time. The increased presence of WNV infections and co-existence with other non-communicable diseases, for which there is also no vaccine available, as well as new communicable diseases such as SARS-CoV-2 in 2020 could pose a variety of pressures to public health authorities.

A synchronous circulation of a diverse set of arboviral infections and the potential of more complex mutations highlights the need for rigorous surveillance in areas where these viruses are active but calls for an improved understanding of the differences and/or similarities in geo-temporal patterns. This is particularly important from a West Nile Virus management perspective as treatments currently available are only

supportive with no vaccine or antiviral medications for humans currently available. Further, considering that WNV human cases may demonstrate symptoms similar to other closely related Japanese encephalitis viruses and/or other infectious diseases, the need to shed light into the specific epidemiological patterns of the disease is vital both for diagnostic and therapeutic purposes.

Finally, WNV disease incidence can be also influenced by climatological conditions which may vary across the continent and respective countries. High temperatures and other hydroclimatic factors may also be contributing to WNV human epidemics. Previous examples have also shown variations and disparities in human clinical symptomology, infection and seasonal peak rates in countries with similar ecologies experiencing viral haemorrhagic-related diseases[5] (Zielinski-Gutierrez and Hayden, 2006). Evidence regarding when, where and to whom outbreaks occur in certain landscape typologies can contribute to a better understanding of the cause of WNV emergence in these areas and may provide an enhanced early warning mechanism to predict and detect future outbreaks.

1.2 Research Objectives

This study is designed to answer three distinctive questions aimed at further informing future policy, research, disease surveillance and other relevant public health preparedness strategies on this topic.

Objective 1: When and where it occurred

[5] accompanied by or produced by haemorrhage caused by weakened blood vessel ruptures.

- Show spatiotemporal occurrence recorded at NUT3 level[6] associated with the occurrence of autochthonous WNV endemic/epidemic outbreaks (rather than sporadic cases)[7];
- Analysis of the territorial typologies (rural, intermediate, urban and/or coastal proximity) associated with human WNV disease outbreaks.

Objective 2: Who is affected

- Explore human epidemiological WNV epidemic trends by age and gender

Objective 3: What are the effects of climate on WNV human disease circulation

- Understand the role of hydro-climatic factors (air temperature, dewpoint temperature, soil temperature, volumetric soil water, wind speed, both eastward and northward, relative humidity, surface pressure and total precipitation) on disease circulation in epidemic stricken countries.

Potential Benefits of this Study

- In addition to its policy implications, information on landscape, demographic and climatic drivers can further assist national and/or regional preparedness efforts.
- It can also serve as a preliminary (baseline) WNV human epidemic model that can contribute to the efforts of developing, based on the European Commission case definition, an EU diagnostic algorithm to further improve the early diagnosis of WNV in human populations.
- It can be used as a geospatial and climatological benchmark exercise to conduct similar EU wide analysis on subsequent epidemics and other

[6] Smallest regional units used for disease diagnoses. More information can be found here http://ec.europa.eu/eurostat/web/nuts/overview

[7] Epidemic refers to "is an outbreak that affects many people at one time and can spread through several communities" whereas Sporadic refers to "a disease that occurs infrequently and irregularly." **Sources:** https://www.cdc.gov/csels/dsepd/ss1978/lesson1/section11.html, page 1. https://www.physio-pedia.com/Endemics,_Epidemics_and_Pandemics#cite_note-:0-1.

mosquito borne infectious diseases for which EU surveillance and climatological data are available.

- The proposed study has the potential to lend support to the European Commission's efforts to identify and address gaps in knowledge and preparedness in EU Member States and its primary recommendation "*to develop a tool for use by public health authorities to conduct risk assessments on the transmission of WNV to humans, using information from multi-sectoral systems*" (ECDC, 2009:1).
- The monitoring of additional parameters and their respective associations, as those proposed in this study, can help predict and detect future WNV outbreaks in Europe, as other studies have demonstrated especially in the US (Sugumaran, Larson and Degroote, 2009; Chen et al., 2008) for which the criteria were relevant to their respective population characteristics.

CHAPTER 2. LITERATURE REVIEW PROCESS

A literature review of scholarly articles and other publicly available sources of intelligence relevant to West Nile Virus was conducted as a means of providing a narrative, synopsis and critical evaluation of these works in relation to the research questions being investigated (objectives 1-3). The review was designed to provide an informative overview of relevant sources of evidence; this assisted with identifying gaps that exist in the literature, interpreting prior research to prevent duplication of research efforts and bringing to light any contradictory or relevant evidence deriving from previous studies.

A literature review was deemed the more suitable form of review as a means of identifying current epidemiological, climatic and environmental knowledge and substantive findings as well as methodological contributions to the study of WNV. A scoping[8] review was deemed unsuitable for a number of reasons. Although in recent years the Joanna Briggs Institute's Scoping Review Methodology Group produced guidance for performing scoping reviews, the purpose for scoping reviews remains unclear with scoping reviews often conducted for purposes that do not align with the original indications as proposed by Arksey and O'Malley (2005) (Peters et al, 2015; Tricco et al., 2015). Munn et al. (2018) provided a number of examples[9] when considering a scoping review approach neither of which were appropriate for this study given that: the vast majority of research is focused on entomological/veterinary studies rather than human epidemiology and the vast majority of climatic and environmental parameters are under researched as the latter have only become available in recent years.

A systematic review, including other associated techniques such as meta-analysis and mixed methods capable of providing an exhaustive summary of current evidence directly relevant to the research questions, was also not suitable. This is because most of the existing evidence was not directly relevant to the key research questions posed

[8] Scoping reviews still lack clarity when defining the exact method as they are still relatively new, so this methodology was also not deemed suitable.

[9] Exemplars for different scoping review indications: To identify key characteristics or factors related to a concept; as a precursor to a systematic review; to identify and analyse gaps in the knowledge base; to examine how research is conducted on a certain topic; to clarify key concepts/definitions in the literature or to identify the types of available evidence in a given field.

by this study to allow meaningful inferences and comparisons to be made. More specifically:

- previous epidemiological research predominantly focused on local entomological/veterinary studies
- research specifically on WNV (cross country) national EU epidemics in human populations was scarce. Most of the human WNV research (89%) focused on regional, rather than national, epidemiological studies.
- the vast majority of published research included all cases including imported rather than autochthonous only and blended endemic and epidemic with sporadic WNV human outbreaks. This is the first cross-country study on EU countries experiencing consecutive outbreaks.
- only some of the nine hydro-climatic conditions researched in this review were (partially and regionally) explored by one other study so evidence synthesis would have not been possible. This is the first, to the author's knowledge, WNV, at multi-country level, study that used ECMWF data.
- there was no single research detected on all five landscape typologies[10] for the NUTS3 areas where human WNV EU epidemics occurred to provide an exhaustive summary of current evidence. This is the first WNV study, to the author's knowledge, that combined ECDC and EUROSTAT typological data of all EU landscape typologies available.

2.1 Literature Review Methods

2.1.1 Databases used

Bibliographic searches were conducted between September 2018 and July 2021 using internet search engines and medical databases as Medline (PubMed) and Scopus; websites of the World Health Organization (WHO), the European Centre for Disease Control and Prevention (ECDC) and other relevant web pages were also searched to ensure that relevant literature was not overlooked. Key references from extracted papers were also searched and included where appropriate.

[10] As defined by Eurostat. **Source:** ec.europa.eu › eurostat › statistics-explained › pdfscache

2.1.2 Search Terms Used

The keywords used for the medical databases were: **“West Nile virus” combined with the following terms:**

Search Cluster 1- **Virology and Disease Management**
or “West Nile fever”, or “West Nile Disease”, or “Flaviviridae”, or “flavivirus”, or “arbovirus”, or “virology” or “genetics” or “Culex”, or “Culicidae” or “mosquito”, “mosquitoes” or “diagnosis”, or “diagnostics”, or “treatment”, or “therapy”, or “prevention”, or “vaccine”, or “control”
Search Cluster 2– **Epidemiological and Clinical**
or “cases”, or “human”, or “transmission”, or “outbreak”, or “epidemics”, or “endemic”, or “epidemiology”, or “molecular epidemiology”, or “gender” or “age” or “male” or “female” or “symptomatic” or “asymptomatic” or “fever” or “encephalitis”, or “meningitis”, or “neurological”, or “surveillance”, or “outbreak”, or “risk assessment,” or “socioeconomic”, or “income”, or “occupation”
Search Cluster 3- **Climate and environment**
or “weather”, or “climate”, or “environment”, or “landscape”, or “territorial”, or “temperature”, or “wind”, or “precipitation”, or “relative humidity” or “rural”, or “intermediate”, or “urban”, or “coastal” or “water”, or “pressure”, or “surface pressure”, or “velocity” “eastward”, or “northward” or “volumetric water content” or “soil temperature”, or “dewpoint temperature”.
Search Cluster 4- **Year** 1996 - 2021
or “1996” … “2021”
Search Cluster 5 – **EU countries**
or “Europe”, or “European”, or “EU”, or “Austria”, or “Belgium”, or “Bulgaria”, or “Cyprus”, or “Croatia”, or “Czech Republic”, or “Denmark”, or “Estonia”, or “Finland”, or “France”, or “Germany”, or “Greece”, or “Hungary”, or “Ireland”, or “Italy”, or“ Latvia”, or “Lithuania”, or “Luxembourg”, or “Malta”, or “Netherlands”, or “Poland”, or “Portugal”, or “Romania”, or “Slovakia”, or “Slovenia”, or “Spain”, or “Sweden” or “the United Kingdom”.

2.1.3 Inclusion and exclusion criteria

The inclusion criteria were:

- All study designs and/or methods i.e., qualitative and/or quantitative, relevant to WNV human cases.
- All European countries/contexts.
- Papers published from January 1996[11] onwards.

The exclusion criterion was:

- Papers not published in English.

No exclusions were made based on study quality. The vast majority of selected studies were descriptive, non-interventional and utilised secondary data at country of regional level.

2.1.4 Study selection

Following screening of all titles and abstracts, full papers which met the inclusion criteria, were selected by the author.

2.1.4.1 Data items, collection, synthesis and summary measures

Papers were grouped into 4 distinct categories:

1. Disease context
2. Epidemiology, demographics and clinical issues
3. Landscape typologies and.
4. Climate factors.

2.1.5 Analysis framework

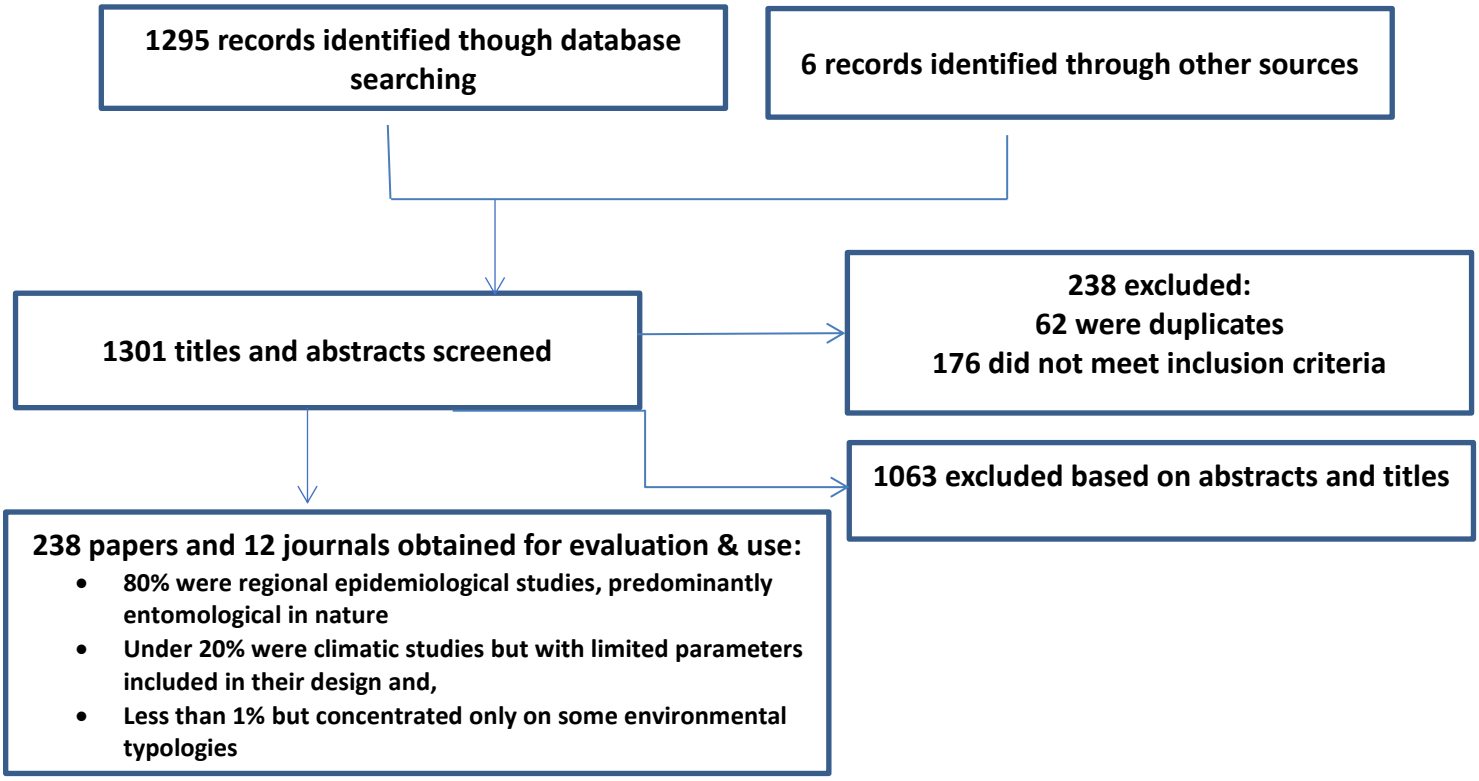
The results were classified into distinctive key areas relevant to the aim of this study based on the results of the search.

[11] Although 1996 to 2009 are not within the scope of this study, they were deemed necessary to be included in the literature review due to their importance given that the first major European outbreak occurred in 1996.

2.1.6 Search Results

Abstracts which met the inclusion criteria were selected based on importance and relevance. **1295** articles were found relevant to human WNV disease of which **238 papers and 12 journals** were relevant to this study.

Flow diagram[12] of selection of reports and articles reviewed.



3. LITERATURE REVIEW FINDINGS

3.1 Disease context

Arthropod-borne viruses (commonly referred to as arboviruses) are viruses transmitted by hematophagous arthropod vectors to susceptible primary and secondary or incidental vertebrate hosts such as humans, livestock and wildlife (Weaver, 2010) during blood feeding (Conway, 2014; Slonchak et al., 2014). Over 550

[12] Flow diagrams utilised in other literature reviews have been adopted in this study i.e., Vašíčková, Veronika. (2020). Crisis Management Process - A Literature Review and a Conceptual Integration. Acta Oeconomica Pragensia. 27. 61-77. 10.18267/j.aop.628.

arboviruses have been detected worldwide (Reusken, 2011; Karabatsos, 1985) mainly carrying mammalian and avian pathogens but around 100 of these are regarded as human infectious agents (Gratz, 2006; Karabatsos, 1985).

Flaviviruses, which include West Nile Virus, is a subset of arboviruses and a genus of viruses belonging to the family Flaviviridae (Lee and Lobigs, 2000). Arthropods tend to multiply near waterholes and/or standing waters during periods of drought (Patz, 2003); following infection they become an amplifying host (Komar, 2003) whereas humans and other mammals are generally considered dead end hosts (Campbell, Marfin and Lanciotti, 2002).

Mosquito-borne flaviviruses (MBFVs) are divided into 2 main groups with over 20 confirmed species, some of which are the most significant vectors of arboviral infections in human populations (Gaunt et al., 2001). *Stegomyia* mosquitoes are highly effective vectors for Yellow fever virus (YFV) and dengue virus (DENV) in various vertebrate hosts whereas *Culex* mosquitoes for West Nile virus (WNV), Japanese encephalitis virus (JEV) and St Louis encephalitis virus (SLEV) mainly in avian species with human serving as dead end hosts.

In general, birds are the amplifying[13] reservoir[14] hosts and ornithophilic mosquitoes (primarily female mosquitoes who acquire, through blood-sucking, proteins from a host to produce eggs) the transmitting vectors (Reusken, 2011). This is particularly the case for West Nile, Sindbis and Usutu viruses (Buckley, 2003). Humans and horses are mainly accidental hosts who do not replicate the virus in the arthropod-vector-vertebrate host viral transmission cycle (Dauphin, 2004). However, some other

[13] Amplifying hosts are "hosts in which infectious agents multiply rapidly to high levels, providing an important source of infection for vectors in vector-borne diseases. **Source**: amplifier host. (n.d.) Farlex Partner Medical Dictionary. (2012). Retrieved September 13, 2020 from https://medical-dictionary.thefreedictionary.com/amplifier+host, page 1.
[14] as a source from which other individuals can be infected.

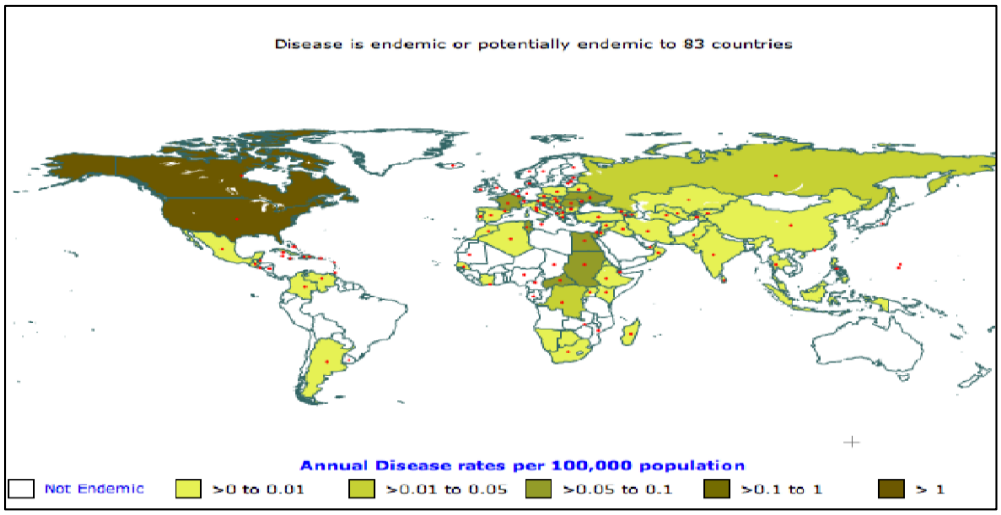
arboviruses, like dengue fever and chikungunya virus, have genetically evolved over the years to such degree that an enzootic circulation is no longer required for the infection to transmit to humans causing widespread epidemics in tropical climates and endemic cases in Europe (Rezza et al., 2007; Weaver and Reisen, 2010; Gould et al., 2010; La Ruche et al., 2010). This reveals a worrying trend regarding WNV's potential evolution and its future containment.

The transmission of arboviruses, through vectors and amplifying hosts, to humans can affect and alter their evolution, mutational adaptation and how infectious diseases spread; these effects are adequately reflected in their phylogenetic relationships (Gaunt et al., 2001). There is also some evidence that human behaviour and activities such as travel, transportation of animals, proximity to livestock and reservoirs and lakes, urbanisation of eco-habitats, excavations and fracking, wars and military interventions, farming and geoponic changes and practices, deforestation and reforestation, pollution and poor building infrastructure and maintenance can trigger the emergence or establishment of flaviviruses including WNV (Gould et al., 2003).

3.1.1 WNV in Europe

WNV, a flavivirus, is by far the most widely distributed arbovirus (Reiter, 2010) detected in many continents (Table 2) including Europe (Rizzoli et al, 2007).

Table 2. West Nile Virus: Global Distribution



Source: https://www.gideononline.com/cases/westnilefever/p1

There is a plethora of evidence that shows that WNV has been continuously circulating in different EU Member States causing local endemic[15] and epidemic outbreaks in several host species including humans (Sambri et al., 2013; Barzon et al., 2012; Sîrbu et al., 2011; Danis et al., 2011). For this and other important reasons, the 7th Framework Programme of the European Union (FP7) invested financial (overall budget €3.9m) and strategic support to shed some lights upon the field (Rizzoli et al., 2015).

Historically consistent surveillance data are not available to compare to present outbreaks; however, the European Centre for Disease Prevention and Control (ECDC) (2014) suggests that there is evidence that the virus has been historically and silently present in Europe. Marcantonio (2015) argues that investigating this arbovirus is of global importance; this is because there are important gaps in knowledge in terms of:

Endemic refers to "*the constant presence and/or usual prevalence of a disease or infectious agent in a population within a geographic area*". Source: https://www.cdc.gov/csels/dsepd/ss1978/lesson1/section11.html, page 1.

- its increasing incidence in many locations across Europe; its ecological patterns and genetic variety
- its increased resistance to many commercial insecticidal solutions
- transferability to and safety concerns for blood transfusion and organ donations (Paty, 2013)
- the risks associated with genetic mutations
- the ability of other mosquito species such as *Aedes atropalpus* interacting with *Culex restua*ns (Turell et al., 2000), and
- the co-emergence of other mosquito borne flaviviruses, such as Chikungunya (CHIKV) and Rabensburg, which may create significant public health pressures for European authorities managing the spread and transmission of infectious diseases (Beltrame et al., 2007; Bakonyi et al., 2006).

3.2 Virology and Pathogenesis

WNV is a single-stranded ribonucleic acid (RNA) of positive polarity and belongs to one of the 67 viruses of the Flavivirus genus which has recently become a public health concern worldwide due to its high mortality and morbidity rates (Mukhopadhyay, Kuhn and Rossmann, 2005; Melik, 2012). It is generally believed that “WNV replicates at the site of inoculation and then spread to lymph nodes and the bloodstream” (Diamond et al., 2003, p.1). Viral infiltration of the central nervous system can lead to stimulation of non-catalytic receptors and play a key role in the innate immune system and increased levels of tumour necrosis factor-α which amplifies penetrability of the blood-brain barrier (Wang et al., 2004). Specifically, WNV attacks neurons found in deep cerebellar nuclei and the substantia grisea of the spinal cord and brainstem (Ceccaldi, Lucas and Despres, 2004; Guarner et al., 2004; Kleinschmidt-DeMasters et al., 2004).

Damage of adjacent nerve cells may be responsible for the loss of muscle function in part of the body (Darman et al., 2004). In some cases, tissue damage caused by the infection may lead to pathologic alterations (Leis and Stokic, 2005). A deficiency in production of 2´–5´-oligoadenylate synthetase in severe neurological disease cases has only been seen in mice so far (Ceccaldi, Lucas and Despres, 2004).

In addition, recent evidence from Austria, the Czech Republic and Germany suggests that around 1% of human population may have been exposed to WNV strains even though there were no human cases detected during 2009-10 in those countries (Rabel et al., 2011). Similarly, analyses of serosurveys performed between 2006 and 2010 and evidence from the 2010 outbreak in Greece showed that the presence of WNV antibodies in humans is as little as 1% (Papa et al., 2010; Krisztalovics et al., 2008). Its precise role, constantly evolving patterns, mechanisms of infection and risks to human public health are neither straightforward nor fully understood.

3.2.1 Genetic Classification

WNV is a genetically diverse virus (De Brackney, 2011). So far, phylogenetic studies on WNV strains isolated from different geographical parts of the world have shown that there are two main genetic lineages associated with WND occurrence in human populations in Europe: Lineage 1 and Lineage 2 (Bondre et al., 2007; Bakonyi et al., 2006; Lanciotti et al., 2002); these contain around 25 to 30% nucleotide variations (Lanciotti et al., 2002) made of numerous subclades, subsets and genetic clusters (Schuffenecker et al., 2005; Venter et al., 2009; Murgue et al., 2001). In Europe, WNV human infections are mainly associated with both Lineage 1 and 2 which cause either asymptomatic, mild febrile, severe or fatal disease (Schuffenecker et al., 2005; Lanciotti et al., 2002).

However, some studies looked at different isolates and found evidence that additional and distinctive lineages exist (May et al., 2011; Bakonyi et al., 2005; Scherret et al., 2001) (Table 3). These have been linked to major WNV outbreaks in humans (May et al., 2011; Vázquez et al., 2010; Botha et al., 2008; Bondre et al., 2007; Bakonyi et al., 2005).

Table 3. Overview of West Nile virus lineages reported in research

Suggested lineage	Other lineage labelling in the literature	Representative strain	Note	References
Lineage 1a	Lineage 1	NY99-flamingo382-99, New York, 1999	Most widespread WNV lineage	Lanciotti et al., 1999
Lineage 1b	Lineage 1	Kunjin MRM61C, Australia, 1960	Kunjin virus strains, Australia	Coia et al., 1988
Lineage 1c	Lineage 5	804994, India 1980	Only found in India	Bondre et al., 2007
Lineage 2	No	B956, Uganda 1937 (oldest WNV strain; WNV prototype strain)	Second most widespread WNV	Smithburn et. al, 1940
Lineage 3	No	Rabensburg virus 97-103, Czech	Only found in central Europe	Bakonyi et al. 2005
Lineage 4a	Lineage 4	LEIV-Krnd88-190, Russia 1998	Originally isolated from Dermacentor ticks	Lvov et al.2004
Lineage 4b	Lineage 6 / Lineage 7	HU2925/06, Spain	Only partial sequence available	Vazquez et al.2010
Lineage 4c	Lineage 9	WNV-Uu-LN-AT-2013, Austria 2013	Identified in Uranotaenia Mosquitoes	Pachler et al.2014
Lineage 5	Lineage 6	Kunjin virus KUN MP502-66, Malaysia 1966	Only partial sequences Available	Vazquez et al.2010
Lineage 6	Lineage 7	DakAr D 5443, Senegal	Koutango virus	NA
Lineage 7	Lineage 8	ArD94343, Senegal 1992	Partial sequence available	Fall et al. 2014

Source : Rizzoli et al.2015 (page 5, adapted table). The challenge of West Nile virus in Europe: knowledge gaps and research priorities. Eurosurveillance, European Centre for Disease Prevention and Control, 2015, 20 (20), 10.2807/1560-7917.ES2015.20.20.21135 pasteur-01659350.

The circulation of other similar viruses and strains such as:

- the chikungunya virus, which was found in human cases in Italy (Mondini et al., 2007; Pialoux et al., 2007; Beltrame et al., 2007),
- a genetic divergent strain found in mosquitoes in Spain,
- the detection of a Koutango viral strain in Senegal and;
- a new Kunjin viral strain identified in Malaysia (Fall et al., 2014; Vázquez et al., 2010; MacKenzie and Williams, 2009; Scherret et al., 2001) may lead to the emergence of new lineages but also further mutations which can pose a risk to European public health. This genetic evolution of the virus suggests that WNV can present significant challenges that make the need to better understand its multifactorial drivers more compelling.

3.3.1 Clinical manifestations

WNV's diverse epidemiological features, genetic versatility and ecology characteristics over the years have demonstrated its capability to cause serious outbreaks worldwide in both human and animal populations. WNV is known to cause mild, non-neurological disease with no apparent clinical cause for the illness or reasons for the presentation of febrile symptoms but also lead to severe central nervous system disease such as encephalitis, poliomyelitis and meningitis (Davis et al., 2004); this broad range covers 20–40% of infected human cases. Research findings show that the vast majority (around 80%) of West Nile Fever cases reported worldwide[16] are broadly either asymptomatic or very mild (Pisani, 2012), whereas the severity of infection, mortality, and morbidity are subject to several conditions including, but not exclusively, the individual's immune status, gender and age. A study has shown that around 1% (in 150 cases) can lead to severe neuro-invasive disease

[16] The precise proportion of these relating to European cases has not been published.

(Xagorari and Chlichlia, 2008). The latter includes meningitis, encephalitis, and acute flaccid paralysis (Kramer, Li and Shi, 2007).

However, recent studies are presenting a more alarming picture with case fatality rates for patients experiencing severe neuro-invasive illness between 4 and 14% across all age groups and as much as 15-29% for the elderly (Petersen and Marfin, 2002); others suggest around 10% mainly for older age groups or individuals with immunocompromised systems (ECDC, 2013). Case fatality rates vary from one epidemic to another with a threefold increase over the years from 4% in Romania (1996) to 17% in 2010, 15% in 2010-11 in Greece (Papa et al., 2013; Danis et al., 2010) and 16% in Italy (2008-11) (Rizzo et al., 2011). These observations are similar to US ranges of 3-15% (CDC, 2013).

As regards gender, due to genetic, hormonal, anatomic, or other differences, men tend to have higher rates of disease mortality and morbidity than women (CDC, 2012). However, the evidence on human WNV on the research questions posed by this study wasmainly available at regional level. A study has also shown that children are generally more likely to develop meningitis over encephalitis compared to adults, tend to require less hospitalisation and have lower mortality rates and neurological symptoms compared to adults (Lindsey et al., 2009).

In addition to age and gender, a variety of socioeconomic factors have also been explored mainly at local/regional level.. Ozdenerol et al. (2008) found that low income was associated with higher WNV infection rates whereas (Gibbs et al. 2006) found that the highest risk was in low housing densities. Rochlin et al. (2011) and Ruiz et al. (2004) reported that high infection rates were mainly associated with suburban middle class, rather than affluent suburbs or the poorer inner cities, neighbourhoods.

Similarly, Harrigan et al. (2010) found that low per capita income, poorly maintained swimming pools and housing types were associated with WNV occurrence. Dowling et al. (2013) and Hribar (2007) studied the presence of larvae[17] in different habitats of different conditions and found larvae in many of the habitat types sampled. Although these results are relevant to the specific region investigated, they indicate that there is a potential link between certain socioeconomic variables and the environment. Alike other studies (Morin & Comrie, 2013; Dowling et al., 2013; Colborn et al., 2010; Patz et al., 2004), Paz et al. (2013) reported that socioeconomic conditions and human behavioural patterns correlated with the spatiotemporal distribution of reservoir hosts and local abundance of mosquito vectors and their capacity to transmit disease.

Many of these studies rely solely on census data regarding socioeconomic information which presents limitations. Studies that combined field data with census data were scarce. Studies that collect primary or secondary data that captures wider socioeconomic characteristics i.e., income and occupation, can yield more universally applicable conclusions. This study utilised secondary datasets that do not collect/include wider socioeconomic characteristics, therefore the latter were not explored. However, socioeconomic variables such as housing density, age and location, property ownership, landscaping, race/ethnicity, income, occupation and education, worth future consideration with regards to their respective effects on WNV human infection rates.

Although the vast majority of infections occur through mosquito bites, disease transmission can be also achieved through organ or blood donation and haematopoietic stem cells, breastfeeding and transplacentally during pregnancy

[17] After mosquito eggs hatch in water, they become mosquito larvae.

(Nanni et al., 2008; Rhee et al., 2011; CDC, 2002; CDC, 2002). In Europe, patient to patient transmission has only been linked to blood and organ transplants where the absence of preventive screening procedures may play a role (Capobianchi et al., 2010). Infections acquired via solid organ transplantation have mainly, but not exclusively, been detected in Italy (Costa et al., 2009; Morelli et al., 2010). Veterinarians and entomologists have also been infected in the past through either laboratory acquired WNV contamination or infections acquired during field surveillance (Venter et al.; 2010; CDC, 2002; Hannoun et al., 1964). Others argue that most human febrile cases of WNV are mild thus rarely require laboratory testing (Kymberly and Gyure, 2009; Gyure, 2009).

Past examples have also shown variations and disparities in human clinical symptomology, infection and seasonal peak rates in countries with similar ecologies experiencing viral haemorrhagic-related diseases (Zielinski-Gutierrez and Hayden, 2006). However, knowledge regarding gender, age and incidence ratios of successive human WNV outbreaks can shed new light on key trends and provide an informative mechanism to respond to future country specific outbreaks.

3.3.2 Diagnostic Issues and Methods

Alike other pathogens, WNV in humans is a notifiable disease in the EU as stated in Commission Decision 2009/312/EC. The case definition for reporting human WNV infections, either probable or confirmed, at EU level is detailed in EU legislation, specifically Commission Decision of 28/IV/2008. Cases are classified as *probable* if they either exhibit (i) pyretic clinical symptoms or (ii) have meningitis or (iii) encephalitis and the presence of specific IgM antibodies in serum or one of the above clinical symptoms and there is an epidemiological link; the latter can be the result of

interhuman (human to human infection through transplanted organs, blood transfusions, or transplacentally) or horizontal (animal-to-human) viral transmission which accounts for the vast majority of cases. Possible cases also require the presence of fever and neurological disease but also specimen testing of acute serum or CSF during illness or convalescent serum shortly after clinical disease subsided (7-14 days post illness). A laboratory *confirmed* case requires an infection to be verified through at least one of the subsequent methods: isolation of WNV from blood or CSF; detection of WNV nucleic acid in blood or CSF; WNV specific antibody response (IgM) in CSF or WNV IgM high titre AND detection of WNV IgG, and confirmation by neutralisation (ECDC, 2008:1).

The presence of viral disease in a (human) specimen is generally identified via either (i) direct detention techniques, (ii) indirect virus isolation methods or (iii) identification of antibodies in the serum and other bodily fluids (ECDC, 2013). Specifically, *direct viral detection* methods examine the presence (or absence) of virus particles, antigen or nucleic acids in a clinical specimen whereas *virus isolation* requires the inoculation of the latter into cell lines, animals or eggs to test for infection by growing the virus inside these cultures; on the other hand, *serological testing* can allow the rapid detection of rising titres of antibody during the critical and recovery stages of viral infection or the presence of specific Immunoglobulin M (IgM) antibodies in cerebrospinal fluid (CSF) or serum (Ford and Rowe, 2004). Given that results from the CSF or the brain can be of greater diagnostic significance than those from other sites, serology has the capacity to be used in laboratory testing settings for a variety of viral infections. There are many different methods available for the laboratory detection of WNV infections (Dauphin and Zientara, 2007). The main diagnostic tests for the detection of WNV available in Europe are described in Table 4.

Table 4. Laboratory tests used for WNV diagnosis and surveillance in humans in the EU

Specimen	Direct virus detection	Indirect Virus Detection
Serum, plasma, CSF and tissue, urine	• Reverse transcriptase – polymerase chain reaction (RT-PCR) • Isolation in cell culture Immunohistochemistry (IHC) • Nucleic acid amplification tests (NAATs) (primarily for screening blood donors)	• Enzyme linked immunosorbent assay (ELISA: IgM, IgG, IgG avidity) • Immunofluorescence assay (IFA) • Seroneutralisation • (Plaque Reduction Neutralisation Test (PRNT))

Source: ECDC, 2008

3.4 Treatment, therapy and prevention

The treatment of WNV-related infections, especially in severe cases, is only supportive at present (Kramer and Shi, 2007), although there is considerable scientific experimentation being carried out, funded by the EU's 7th Framework Programme, which uses high-throughput screening methods and seeks to identify active compounds that modulate WNV's specific biomolecular pathway.

In severe West Nile encephalitic disease (WNED) cases patient recovery can be optimised if hospitalisation in an intensive care unit and provision of life support therapy, followed by rehabilitation, are prioritised (Kramer and Shi, 2007). For infections acquired via solid organ transplants or haematopoietic stem cells or blood transfusion, large doses of commercially available hyperimmune plasma products or purified immunoglobulins with elevated titres of antibodies against WNV have been administrated in the recent past (Rhee et al., 2011; Morelli et al., 2010).

When patients exhibit meningeal symptoms, pain killers are administered and drugs to alleviate nausea, vomiting and dehydration. For cases with severe encephalitis, medical care includes the monitoring of raised intracranial pressure and seizures;

patients with encephalitis or paralysis require oxygenation. Given that respiratory failure may develop rapidly in severe cases, particularly in patients depicting an impairment of function of certain cranial nerves, prolonged mechanical ventilation may be necessary (Kulstad and Wichter, 2003; Sejvar et al., 2003; Leis et al., 2003).

3.4.1 Preclinical approaches to West Nile vaccine development

The development of a safe candidate vaccine against WNV for animals and humans has been the focus of many studies (Wang et al., 2003). At present, there have been a number of attempts to develop a West Nile vaccine including DNA-vectored, live chimeric/recombinant vaccines and live attenuated or pseudo-infectious vaccines as well as recombinant subunit vaccines and inactivated whole virus vaccines (Amanna and Slifka, 2014). Some of these experimental platforms include: *formalin-inactivated vaccines* which have been used for the protection of laboratory workers and high risk groups (Pittman and Plotkin, 2008), the use of *recombinant WNV proteins or recombinant E protein* which yielded positive results in animals (Lieberman et al., 2009), a suggested *single dose of defective viral particles* which appeared to be cost-effective and clinically effective in immuno-compromised cases (Widman, Frolov and Mason, 2008) and *attenuated vaccines* which can be reasonably cheap to produce, potent, and safe but limited in terms of its safety for immuno-compromised subjects.

However, long-term immunogenicity and safety remains a vital parameter in the experimental development of candidate vaccines against WNV, especially for lineages 1 and 2 which has been the focus of many European consortia. The potential risk of chronic kidney damage in children and younger human populations (Nolan et al., 2012) and chronological persistence of disease identified in urine samples of recovered subjects (Murray et al., 2012) make the need for an efficacious human

vaccine against WNV disease more pressing. For patients with Guillain-Barré syndrome, non-specific immunoglobulin and plasmapheresis can be administered but these are not suitable for those with motor paralysis (Sejvar et al., 2003). Further, in the case of WNED, long-term health problems, i.e., muscle weakness and memory problem, can occur therefore surveillance and prevention can be more important tools than disease therapies.

3.4.2 Antimicrobial Resistance

Additionally, secondary infections such as pneumonia or urinary tract infection may require the use and administration of antibiotics. However, Gendrin et al. (2015) claim that in the case of malaria, another vector borne disease, the use of antibiotics by humans can affect the mosquito microbiota and increase disease transmission. Research suggests that *Culex sp*., the primary vector for WNV, are also involved in Malaria transmission in southern Europe (Sainz-Elipe et al., 2010).

3.4.3 Prevention

Certain protection measures have been found to be effective in preventing vertical transmission of WNV infections; these include repellents containing 50% diethyltoluamide (DEET), avoiding stagnant pools of water where mosquitoes breed, using insect-proof screens and mosquito nets, wearing appropriate - light and skin covering- clothing (NHS Choices, 2013). However, within Europe, monitoring and surveillance systems regarding the circulation of WNV in humans vary (Table 5) from country to country (Sambri et al., 2013). Given that surveillance, public outreach

campaigns, disease containment strategies, mitigation and response in the event of WNV epidemic outbreaks is a national competence, there is currently no central EU policy on WNV prevention, apart from the ECDC's risk management tools (ECDC, 2015).

In addition, the ECDC collects and publishes EU surveillance data and some geographical information on WNV-affected countries[18] in the form of tables and interactive geospatial maps. However, the former were initially periodic. For example, there were no ECDC WNV data published in 2010. The production of consecutive, annual maps on disease burdened EU countries can produce a better understanding of disease hotspots and potentially influence future planning. Further, although general annual maps are a valuable tool in terms of disease containment of both horizontal and vertical transmission of WNV disease in humans, a focus on epidemic stricken countries can further inform their respective public health responses. Such spatiotemporal maps have the potential to become a powerful disease prevention European asset if retrospective longitudinal data is presented spatiotemporally and supported by other climatic and environmental drivers**.**

The potential of the WNV to cause severe disease in humans and animals (Campbell et al., 2002) highpoints the need to have in place robust public health and veterinary early warning systems. Following the Romanian epidemics in late 1990s and other country level endemics, WNV specific entomological, animal, and human surveillance programmes for WNV have been implemented in a number of EU countries (Barzon et al, 2013; Valiakos et al., 2011; Calistri et al., 2010; Jourdain et al., 2008).

[18]available at http://ecdc.europa.eu/en/healthtopics/west_nile_fever/West-Nile-fever-maps/pages/index.aspx

For example, some EU neighbouring countries (Albania, Kosovo and Montenegro) monitor disease only in human populations whereas other EU and neighbouring countries (Hungary, Italy, Greece, Germany, United Kingdom, Spain and Serbia) follow an interdisciplinary approach to identify information from equines, avian species humans and mosquitoes (Barbi et al., 2013; Marka et al., 2013; EpiSouth, 2012) to detect disease circulation at the earliest possible stage. Italy's interdisciplinary approach covers extensive WNV veterinary, entomological, blood and organ donation and human case surveillance (Rizzo et al., 2016); Greece also implements an active, case tracing, surveillance programme (Table 5). Specifically, in October 2011 Greece put in place the *"Integrated surveillance programme which monitors West Nile virus and malaria in Greece"* (MALWEST)[19] (Marka et al., 2013, page 1). Considering that WNV circulates seasonally in many EU countries, the main aim for a cross-country comprehensive surveillance system is the prompt discovery of viral presence during the vector season to allow for preventative public health measures to be put in place (Calistri et al., 2010).

Table 5. Key characteristics of West Nile virus (WNV) infection surveillance in the European Union, Greece and Italy

Country/region	Intersectoral collaboration	Human	Animal	Vector
European Union	ECDC provides WNF seasonal maps that include human cases and provides information on	WNF is an EU notifiable disease; cases are reported by EU countries to TESSy according to	WNF, as a cause of equine encephalomyelitis in horses, is notifiable to the European Animal Disease Notification System. WNF in animals is	There is no legal framework regarding mosquito surveillance at EU level.

[19] "The aim of the project is the development of an Integrated Control programme related to West Nile virus (surveillance for human cases, mosquito surveillance, avian surveillance, and equine surveillance) and malaria (surveillance for human cases, mosquito surveillance). The main objectives of the programme are: 1) the detection of the West Nile virus and malaria plasmodium activity and their impact on public health, 2) the detection of the geographic regions with the greatest risk and the development of risk assessment tools by using Geographical Information Systems (GIS), 3) the prediction of spreading of the disease and 4) the assessment of appropriate interventions".. Source: http://www.malwest.gr/en-us/nsrf.aspx, page 2.

Country/region	Intersectoral collaboration	Human	Animal	Vector
	animal/vector WNV infection. ECDC tool for WNF risk assessment proposes a classification of risk areas based on human, animal and vector surveillance data. EFSA and ECDC publish the EU summary reports on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks every year.	the EU case definition. EU countries must implement a deferral of blood donations for 28 days after leaving an area with ongoing WNV transmission in humans.	a disease listed in the OIE Terrestrial Animal Health Code and must be reported to the OIE. EU countries should monitor WNV activity in animals, if warranted by the epidemiological situation, and report animal cases to the European Commission.	ECDC developed guidelines for surveillance of native mosquitoes to support countries to plan and implement surveillance activities.
Greece	The Ministry of Rural Development and Food and the HCDCP share the results of human, animal and vector surveillance with each other and with regional and local public health authorities, local veterinary services, municipalities and local health units There are a multisectoral committee for the prevention and management of	Since 2010, all laboratory probable and confirmed WNF cases should be notified. Enhanced surveillance is implemented at national level that includes awareness campaigns towards physicians, support of laboratory confirmation, active laboratory-based surveillance,	WNF in animals is a notifiable disease and disease suspicions must be reported to the competent veterinary authorities. There is active serological surveillance of sentinel horses; active clinical surveillance of equidae around confirmed human and animal cases; passive surveillance of WNF in equidae all-year-round and some small-scale surveillance in wild birds.	Since 2010, HCDCP together with the National School of Public Health, Universities, local authorities and subcontractors, conducts active vector surveillance from June to October to detect WNV circulation in mosquitoes.

Country/region	Intersectoral collaboration	Human	Animal	Vector
	tropical diseases (including WNF) and two multisectoral working groups: for vector-borne diseases and for the designation of areas affected by such diseases.	cases investigation and daily dissemination of information to national and local stakeholders. Measures for the safety of blood products are taken in line with the EU directive for blood safety.		
Italy	A national plan for surveillance on imported and autochthonous human vector-borne disease (chikungunya, dengue and WND) integrating human and veterinary surveillance is prepared annually. Any suspected evidence of virus circulation in animals or vectors is notified to the public health authorities.	Probable and confirmed human cases are notified in real time using a password-protected web-based system. In the affected area, local health authorities implement an active surveillance at risk population. Passive surveillance on human neurological cases is set up in the whole region where the affected area is located. Measures for the safety of blood products are taken in line with the EU directive for blood safety.	A web-based national animal disease notification system allows the notification of animal diseases. Passive surveillance in equidae in the whole country. ▪ Random IgM screening in horses living in non-affected areas. Bird surveillance focuses on WNV detection in resident target species; immunological response among poultry of rural farms and migratory birds; and bird mortality.	Entomological surveillance is systematically implemented during the period of vector activity in affected areas.

Source: Gossner et al., 2017, page 3.

An active surveillance of migratory bird populations, whereby authorities proactively look for presence of disease, can provide valuable information regarding the locations that may be at risk of WNV circulation but is less informative about the threats to public health. On the other hand, many EU countries, including Italy, France and Greece, have placed great emphasis on actively monitoring the potential circulation of the virus in resident birds (such as magpies and carrion crows) (Valiakos et al., 2011; Calistri et al., 2010; Jourdain et al., 2008). Finally, proactively tracing the virus through mosquito surveillance is rare as its circulation can be diluted and difficult to trace where large pools of insects gather and cohabit. The need to improve the capacity of detecting the virus from mosquitoes has been the subject of ongoing studies (Hall-Mendelin et al., 2009). In Italy, Greece and Romania, where WNV is recurrent year on year, human WNV disease at regional level has been explored (Rizzo et al., 2012; Ceianu et al., 2001; Danis et al., 2011) albeit not consistently across years and all affected regions.

The passive surveillance of dead birds has been widely used in many European countries (ECDC, 2012). However, the WNV strains circulating in Europe, in contrast to those circulating in the Americas with alarming consequences in bird mortality (Martín-Acebes and Saiz, 2012), do not lead to significant mortality rates in birds; therefore, its continental effectiveness and usefulness is unclear in terms of detecting the presence of WNV in Europe. As regards the passive surveillance of horses, scientists have looked at morbidity and/or mortality rates in conjunction with cross-sectional surveys or in some other cases the use of sentinel animals tested repetitively during the vector season (Garcıa- Bocanegra et al., 2011; Calistri et al., 2010). Other

studies suggest similar combinations of surveillance programmes for horses but also sentinel or wild resident birds (Chaintoutis et al., 2014; Calzolari et al., 2013; Rizzoli et al., 2007; Figuerola et al., 2007). Additionally, considering that WNV is not a contagious disease, passive surveillance in humans may have limited value in terms of providing an early warning that the virus is in circulation. A potential addendum to integrated surveillance approaches would be to disinter early trends of hydro-climatic parameters affecting human WNV outbreaks.

3.5 WNV Distribution

Following its isolation in 1937 and up to the 1950s, West Nile Virus was only detected in territories of the Old World, mostly in the temperate areas of Africa, Israel and India (Calistri et al., 2010). It gradually expanded to new and Old-World countries such as Egypt (1950s), France (1960s) and South Africa (1970s) (Zeller and Schuffenecker, 2004; Murgue et al., 2001; Hubálek and Halouzka, 1999).

From 1994 onwards, neuroinvasive WNV was reported in many countries outside the EU, for example in Algeria (1994) (Reiter, 2010), Russia (1999) (318 human cases; 40 deaths) (Platonov et al., 1999). From 1999 to 2012, the United States of America which witnessed several epidemics with over 29,000 human cases being recorded, over 10% of which have led to death. The largest epidemic in the history of the country took place between 2002 and 2003 in California (CDC, 2008).

WNV is now one of the most spatially spread arbo-pathogens in the world with human infections recorded in almost all continents (Kramer, Styer and Ebel, 2008) including Europe, where infections were initially sporadic and mainly reported due to either

transport of infected humans or birds (Papa, et al., 2010; Rizzo et al., 2009; Krisztalovics et al., 2008).

Although a few studies have shown that WNV infections may be found only in some vertebrate hosts (Ceccaldi, et al., 2004; Kuno; 2001), the emergence of WNV infections in humans in the same area in successive years may suggest that the virus is permanently established (Kalaycioglu et al., 2012).

3.5.1 WNV human infections in the European Union and neighbouring countries

Alike the United States, the number of European countries reporting WNV activity and related outbreaks has increased considerably over the years (ECDC, 2012). This is particularly important as vaccines to prevent WNV-related infections are not available.

Since its re-emergence in the EU in 2010, which caused endemic and epidemic outbreaks in Italy, Romania and Greece (ECDC, 2014), surveillance efforts have increased which may contribute to the higher rate of incidence recorded; this fact poses an interesting question as to whether the virus was silently present before that period but remained undetected due to reduced surveillance (Table 6).

Table 6. Selected Reports of West Nile Disease Outbreaks in European Union Countries prior to 2010.

Country	**Year**	**Species Involved/Clinical Symptoms**		
		Human	**Equine**	**Birds**
Austria	2008			NK
Croatia	2001-2002		No	
Czech Republic*	1997	Yes		
	2004-2006			No

Country	Year	Species Involved/Clinical Symptoms		
		Human	Equine	Birds
France*	2000		Yes	
	2003-2004-2006	Yes	Yes	
Greece	1970-1980		No	
Hungary	2003-2008	Yes		
Italy*	1998	No	Yes	No
	2008-2009	Yes	Yes	No
Poland	2006			No
Portugal	1971			
	2004	Yes		
Romania	From 1996	Yes	Yes	Yes
Spain	2003-2005			No
	2004	Yes		

Source: Calistri et al., 2010. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2878979/, page 2. Notes: Yes: presence of clinical symptoms; No: absence of clinical symptoms; NK: not known. *WNV detected in mosquitoes.

Following on from the severe epidemic outbreaks in Bucharest in 1996 (393 hospitalised cases; 17 deaths) (Hubalek and Halouzka, 1999), there has been a substantial increase in West Nile infections in humans across the European continent (ECDC, 2012) including in France, Greece, Hungary, Italy, Romania, Spain and the United Kingdom (ECDC, 2014). For example, 261 human WNV cases were reported in Greece in 2010, 101 in 2011, 161 in 2012 and 86 in 2013 with 42 deaths recorded in the first two years only (EpiSouth Network, 2013; ECDC, 2013; Barzon et al., 2013; EpiSouth Network, 2012; Rizzo et al., 2012; García-Bocanegra et al., 2012; Papa et al., 2012; Valiakos et al., 2012; EpiSouth Network, 2011; García-Bocanegra et al., 2011; Papa et al., 2011; Valiakos et al., 2011; Sirbu et al., 2011; EpiSouth Network,

2010; World Organisation for Animal Health, 2010; ECDC, 2010). The above clearly demonstrates an important change in the epidemiological situation at least in some EU countries and a gap in terms of our knowledge on the epidemiological profiles of those EU countries experiencing recurring endemic or epidemic outbreaks.

In 2010, Greece has endured a significantly large outbreak of WNV infections with around 70% of cases developing neuro-invasive disease and 12% fatalities (Danis et al., 2010). Since 2010, Greece has been experiencing on-going waves in its Central, Northern and Southern regions for two consecutive years with a relatively high case fatality rate (17%) (ECDC, 2012).

3.6 Climatic and Environmental Conditions Affecting WNV Circulation

Historic evidence shows that over the decade ending in 2010 many European countries experienced one of the fastest climate warming trends, especially countries in Southern and South-eastern Europe which experienced significant heat waves and elevated temperatures (WMO, 2010). These trends may have contributed to the occurrence of the 2010 epidemic of WNV in human populations in Greece. Climate warming has the potential to lead to a rise in numbers of mosquito populations, which can facilitate the formation of new, more severe viral mutations and result in arboviral diseases being permanently established in many diverse landscape typologies. If WNV was to permanently establish itself outside its traditional tropical areas this may have broader and more severe consequences on public health considering that the treatment of WNV is only supportive. Thus, a better understanding of the role of both climate and environment in WNV epidemiology is clearly needed.

3.6.1 Territorial typologies

Surveillance exercises conducted on marshlands from 2001 to 2011 detected the presence of WNV in many locations in Greece and Italy (Chaskopoulou, 2011; Filipponi et al., 2008; Filipponi et al., 2005). Their findings support the statement that there is a correlation between WNV circulation and certain ecosystems, where birds are known to migrate to and settle (Figuerola et al., 2007; Parreira et al., 2007; Malkinson et al., 2002).

In addition to wetlands, densely populated humid urban and industrial areas have also been found to be involved in WNV outbreaks with disease prevalence increasing near areas which attract mosquitoes such as leaking pipes and standing water (Schvoerer et al., 2008). However, other observations showed that WNV may also operate as a "silent disease" and circulate in certain areas of North-Eastern Italy for two successive years, with no record of either neuroinvasive or non neuroinvasive disease transmission in accidental hosts (Calistri et al., 2009).

Ruiz et al. (2007) and Ruiz et al. (2004) found that WNV manifestation was noticeably higher in inner urban areas of Chicago and Detroit. Although WNV outbreaks in Romania, Russia and the United States in the late 1990s were closely linked with urban environments, most epidemics following the initial isolation of WNV, were traced in rural areas (Hayes, 2001). A variety of studies found associations between WNV presence in birds, humans and horses and both rural and urban areas across Europe (Paz et al., 2013; Paz and Semenza, 2013; Kilpatrick et al., 2008; Hamer et al., 2008; Turellet al., 2005; Ruiz et al., 2007; Dohmet al., 2002; McLean et al., 2001).

Others have observed a close association between the coastal regions of Israel and WNV cases in humans (Bassalet al., 2017; Anis et al., 2014). Similarly, Kalaycioglu et

al. (2014) and Ozkulet al. (2013) reported that the Mediterranean coastal zones of Turkey were more susceptible to WN disease outbreaks. The role of urban, rural, intermediate and/or coastal areas in WNV transmission requires further analysis and a better understanding of whether the presence of the disease is associated with certain territorial typologies.

3.6.2 Climatic factors

Evidence suggests that birds who are infected in the winter in Africa migrate to other continents during the spring season; this explains the reasons why WNV is detected in Europe during spring with human outbreaks taking place during the summer period (Rappole and Hubalek, 2003). Certain climatological factors (Rosalie & Bisesi, 2020) such as humidity and precipitation have been explored (Semenza and Menne, 2009, Paz, 2006; Patz, 2005), with air temperature the primary focus of predominantly national and/or regional WNV research. The full role of meteorological conditions in the circulation of this emerging arbovirus in Europe is not fully understood.

3.6.2.1 Ambient temperature

A number of studies have demonstrated that air temperature is a key factor for viral replication and transmission of WNV in human populations (Paz et al. 2013; Andrade et al., 2011; Kilpatrick et al., 2008; Reisen et al., 2006; Kinneyet al., 2006). An increase in vector populations has been linked to high air temperatures (30 °C) (Paz and Albersheim, 2008). Further, research shows that elevated temperatures reduce the time required for blood meals and expedite the pace of virus transition (Paz et al., 2013; Ruiz et al., 2010; Kilpatrick et al., 2008; Meyer et al., 1990).

In an experimental study, Dohm and Turell (2001) found that the vast majority of *Cx. pipiens* mosquitoes were infected at 26 °C but none at lower temperature records. In a subsequent study Dohm et al*.* (2002) discovered that nearly all *Cx. pipiens* mosquitoes tested positive for WNV at 30 °C within 4 days of acquiring the infection but 25 days after at 18 °C. Kilpatrick *et al.* (2008) established that there is a positive association between temperature and time and *Cx. pipiens* infections in the dispersal of certain genotypes of WNV. A modelling study confirmed that high temperatures were linked with WNV distribution and viral presence was detectable within 7 days (Ruiz et al., 2010). A number of studies support the hypothesis that elevated temperature play a critical role in the transmission of WNV (Paz*,* 2006; Pats, 2003; Dohm et al*.,* 2002; Epstein, 2001; Cornel et al*.,* 1993). It was reported that a moderate increase of ambient temperature may trigger elevated WNV transmission rates (Kilpatrick et al*.,* 2008).

Although the virus has an ability to reproduce under a wider range of temperatures (as low as 14 °C in poikilothermic mosquitoes (Cornel et al., 1993) to 45 °C in symptomatic avian hosts (Kinneyet al., 2006), higher transmission rates are generally associated with higher temperatures (Jia et al., 2007; Kunkel et al., 2006). However, it is noteworthy that very high temperatures over 30 °C can slow WNV activity in certain mosquito species notably Cx. *Univittatus* (Reisen et al., 2006).

Studies from the US, Israel and Europe suggest that a preceding hotter summer can trigger endemic outbreaks in the region in the subsequent summer (Lourenço et al, 2020; Paz et al*.,* 2013; Paz*,* 2008; Paz and Albersheim, 2008; Reisen et al*.,* 2006; Epstein, 2001). Similarly, Reisen et al*.* (2006) indicated that WNV tends to spread into new locations when summer temperatures are higher than average. This may explain

the 2011 and 2012 WNV outbreaks in humans in parts of Europe and Eurasia which followed a local pattern similar to the disease transmission observed in 2010 (Paz and Semenza, 2013).

Based on the World Meteorological Organization (WMO), 2010 was one of the hottest years ever recorded in Europe with successive very hot nights recorded across the continent and surrounding areas (WMO, 2012) with Russia, Romania and (to lesser degree) Greece experiencing severe heat waves (from >3 °C to >9 °C above the 30-year average mean) (Paz et al., 2013). Considering that an estimated rise of 1.4-5.8°C in human generated by gas emissions has been predicted by 2100 (IPCC, 2007), global warming may present additional challenges and healthcare pressures for public authorities, healthcare personnel and the general public. Paz et al. (2013) observed that even in northern European countries, i.e., Germany and Austria, temperature had a significant positive association with WNV manifestation in humans (within or up to 4 weeks) in that year whereas in southern European countries it correlated without any lags. Favourable temperatures for virus amplification in mosquitoes were also observed in previous WNV outbreaks (Paz, 2006; Semenza and Menne, 2009; Savage et al., 1999).

3.6.2.2 Dew point and soil temperature

DeGroote et al. (2008) have previously observed a strong connection between dew point temperature and disease distribution (before, during and after an infection occurred) but Soverow et al. (2009) only found a link after an infection had occurred. Stilianakis et al. (2016) identified soil temperature as a key factor influencing WNV distribution in Northern Greece. This parameter remains scarcely researched.

Overall, no research was identified looking collectively at the impact of air temperature on European countries experiencing consecutive WNV outbreaks following the 2010 surge. Other forms of temperature such as soil temperature and dewpoint temperature have been inadequately considered and the findings are neither homogenous nor conclusive.

3.6.2.3 Wind

The role of wind on WNV spread has not been extensively researched. Mackenzie et al. (2004) suggest that there may be an indirect link between wind and disease dispersal whereby the former is assisting the migration of mosquitoes. Similar observations were reported on *Cx. tritaeniorhynchus* mosquitoes in China in an earlier study (Ji-Guang and Mei, 1996) and more recently on storm-driven birds (Paz and Semenza, 2013; Bengtsson et al., 2006).

Stilianakis et al. (2016) found that low wind velocity can be linked to higher incidence of WNV infected mosquitoes and emergence of human WNV cases, but high wind was associated with lower human WNV transmission. On the other hand, Yasuoka and Richard (2007) reported negative associations with the presence of WNV. Overall, the role and types of wind including direction i.e., eastward, as a dispersal factor in WNV outbreaks in human populations requires further investigation.

3.6.2.4 Total precipitation

In addition to temperature, the impact of precipitation on WNV infections has long been researched (Ciota and Kramer, 2013; Lake et al., 2012; Paz and Semenza, 2013). The role of precipitation in WNV outbreaks in humans, mosquitoes and reservoir species has been more multifarious but not consistent across studies (Wimberly et al., 2014;

Crowder et al., 2013; Chung et al., 2013; Walsh, 2012; Deichmeister and Telang, 2011). Higher WNV incidence rates were detected when rainfall levels in the preceding year were higher (Landesman et al., 2007) and lower in the year (Hahn et al., 2015). Precipitation is likely to interact with temperature given that dry periods play a role in WNV transmission (Shaman et al., 2005; Paull et al., 2017). Increased WNV activity has been observed during March when weather is warmer and dry, but this evidence is inconclusive as other antithetic parameters including wet conditions have been found to affect WNV circulation (Little et al., 2016).

On the other hand, Ukawuba and Shaman (2018) identified limitations with using precipitation rather than overall water retention by soil to predict WNV outbreaks; the authors concluded that soil moisture and temperature act as biggest predictor of WNV occurrence with rainfall being a comparatively less important factor.

3.6.2.5 Relative humidity

Relative humidity (RH) is "*the ratio of the partial pressure of water vapour to the equilibrium vapour pressure of water at the same temperature near the surface*" (ECMWF, 2016, p1). Hess et al. (2018) suggested that humidity influences the survival of adult mosquitoes and their behavioural pattern but no direct links to human disease occurrence. Stilianakis et al. (2016) found that high WNV incidence correlated with low values of relative humidity.

Two (2013) studies identified a general association between human WNV disease and weekly relative humidity in the Europe basin, but disease outbreaks were less clearly associated (Paz et al. 2013; Paz and Semenza, 2013). Paz (2006) reported a positive link between WNV hospital admission cases in the Tel Aviv area and RH values. On

the other hand, Mulatti et al. (2014) stated that humidity was not a strong predictor in mosquito replication rates suggesting a limited role in the former causing human outbreaks. The effects of relative humidity in WNV circulation are not clear (Paz, 2015) and remain variable (Lebl et al., 2013).

3.6.2.6 Surface pressure

Reisen et al. (2004) reported that high atmospheric pressure in Nevada during the summer of 2003 was the likely cause of emergence of WNV in Colorado, South-eastern California, Arizona and northern Mexico. Its role in WNV spread may be facilitative as during drought increased mosquito amplification can occur as a result of reduced natural predators (Chase, 2003) and therefore create a supporting environment for larval incubation. However, surface pressure remains considerably under researched in the context of WNV outbreaks.

3.6.2.7 Volumetric Soil Water Content

As regards Volumetric Soil Water content previous studies have shown that the underlying groundwater levels are capable to better estimate the overall role of water in disease incidence, which is often influenced by other climatic parameters, e.g., temperature, wind velocity as well as landscape typology (Rodell, 2017; Stilianakis et al., 2016; Shaman et al., 2002). The ability of soil water content and other pertaining factors such as surface pressure to become WNV predictors needs further exploration.

SUMMARY STATEMENT/CONCLUSIONS

The above review identified a number of gaps in knowledge which the proposed study will aim to explore for those European countries experiencing consecutive WNV

outbreaks during the suggested study period. The following points synopsise the key gaps in literature and benefits of the present study.

- ECDC published data on human WNV transmission do not concentrate on countries experiencing consecutive outbreaks. The production of focused and consecutive EpiMaps of WNV burdened EU countries based on retrospective longitudinal data can produce a better understanding of disease hotspots as well as aid future epidemic outbreaks, prevention and containment efforts.
- The vast majority of research on the topic in the EU is dominated primarily by epidemiological studies, mostly regional in nature. Analysis of country demographic patterns (in terms of age and gender) can provide useful epidemiological information for at risk groups in those countries experiencing successive epidemic outbreaks.
- This review showed that WNV can spread and establish itself outside its traditional tropical areas. However, there was no clear country evidence to define whether WNV epidemic stricken areas are rural, intermediate, urban or coastal. Although some research on rural and urban associations exists, it is not consistent throughout the studied years and across countries experiencing consecutive outbreaks. Research that considers intermediate and coastal links to WNV transmission is almost non-existent.
- In terms of climatological factors, although humidity and precipitation are relatively well researched (Semenza and Menne, 2009, Paz, 2006; Patz, 2005) with air temperature the focus on many national studies (Stylianakis et al., 2016; Paz et al., 2013) more is needed to understand their temporal effect on human WNV outbreaks. Other possible contributing meteorological factors such as dewpoint temperature, soil temperature, volumetric soil water, wind

components and surface pressure have been heavily neglected at national level and/or EU level.

Investigating the above areas can allow the unpacking of certain characteristics of European populations and environments experiencing epidemics. This approach has not been previously explored in European research, specifically combining epidemiological data from the European Centre for Disease Prevention and Control (ECDC), landscape data (Eurostat) and climatological data recorded by the European Centre for Medium-Range Weather Forecasts (ECMWF). It aims to create a meaningful picture of why certain countries are experiencing, almost consistently, WNV epidemics.

CHAPTER 4- METHODS

This chapter makes use of data from three distinctive sources:

1. Historic case-based data extracted from the Surveillance Atlas of Infectious Diseases, which is based on ECDC's European Surveillance System (TESSy), and directly, through a paper-based data collection, from the ECDC for the period between 2010 and 2015; the latter collects Member States data at NUTS 3 level on human WNV infections.
2. The second part combined ECDC data with Eurostat data on rural/urban/intermediate typologies as well as coastal approximation to affected areas.
3. The third part of this study utilised historic climatological data recorded by the European Centre for Medium-Range Weather Forecasts

(ECMWF) at NUTS 3 level combined with human WNV infections notified at EU level to the ECDC's TESSy.

4.1 ECDC DATA

The first part contains a descriptive epidemiological report, based on human WNV infections notified at EU level to the ECDC using relevant territorial units at NUTS 3 level. WNV infection is a notifiable disease at EU level (ECDC, 2016) and its primary sources are tertiary healthcare facilities (i.e., hospitals) and laboratories based on probable and confirmed human cases of WNV infection according to the EU case definition[20]. The data capture autochthonous, interhuman (SoHo), travel and vertically acquired cases. Asymptomatic cases not reported in those settings are not captured in the ECDC data. Case-based data were extracted from the ECDC's European Surveillance System (TESSy) on human WNV infections for the period between 2010 and 2015. ECDC's confidentiality forms were submitted in July 2018. The primary fields contained in this dataset included the number of cases, date of infection reported, NUTS3 location of infection and/or reporting, male/female cases, age and country. All data are secondary and anonymised prior to analysis. The research proposal was submitted to and approved by the ethics committee of the health research division of Lancaster University.

Data on human WNV cases are reported yearly by all EU Member States and three EEA countries (Iceland, Liechtenstein and Norway) from their respective surveillance systems to ECDC. Data and metadata include case characteristics in accordance with the case definitions established by the EU (European Commission, 2002). An

[20] West Nile virus risk assessment tool - ECDC - Europa EU. Source: www.ecdc.europa.eu › files › publications › Publications

additional (event based) surveillance process, using direct reporting by Member States, is employed to further inform the annual TESSy data collections, through the EC's Early Warning and Response System (EWRS) and the Epidemic Intelligence System (EPIS). Given that Member States are legally required to report these data, it can be confidently assumed that datasets are complete and representational. The study also explored country and region level data held by the European EpiSouth[21] and EpiNorth[22] Networks, which are ECDC funded regional collaborative frameworks on epidemiological issues,

However, a test-retest was carried out where ECDC data were missing or different compared to online reports; this was checked against the ECDC funded EpiSouth and EpiNorth Network reports produced by the affected countries (pilot test), and ECDC's (event based) surveillance data. This ensured that study findings were based upon up-to-date and reliable data.

The data used in this report were retrieved from TESSy as of 12 August 2017. No subject recruitment was required for this study. The study was neither experimental nor interventional in nature. It utilised anonymised, secondary data, the vast majority of which is in the public domain.

The inclusion criteria for the present investigation were: all reported, probable and confirmed, cases of all ages and both genders within countries of the European Union (EU28) (namely Austria, Belgium, Bulgaria, Cyprus, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia,

[21] Network for Communicable Disease Control in Southern Europe and Mediterranean Countries Source: http://www.episouthnetwork.org/
[22] Network for Communicable Disease Control in Northern Europe. http://www.epinorth.org/

Slovenia, Spain, Sweden and the United Kingdom) between January 2010 and December 2015. Data prior to 2010 were neither published nor routinely collected by the ECDC in the current format. As data access requests were made in 2016, data only up to 2015 was made available. The sample size was a total of 1,330 human WNV cases across the EU.

Imported cases, such as infections acquired outside the EU but reported in an EU country, were subsequently excluded in the analysis as infections were not locally acquired. Disease acquired via blood transfusion and transplantation were also not included in the sample as they are spatially difficult to determine. The study did not differentiate between human WNV neuroinvasive and non-neuroinvasive infections.

Spatiotemporal analysis for epidemic stricken countries was conducted on the ECDC's European Map Making Application (EMMa) based on ECDC and Eurostat (population) data (2010). The ECDC datasets, which included WNV cases, age, gender and locations, were subsequently combined with the following variables: place of infection (using Eurostat's NUTS-3 levels) (European Commission, 2017), levels of urban/rural/intermediate classification based on population distribution and time. For 4% of the reported cases, alternative dates were used: date of notification (3.6%) and date of diagnosis (0.4%).

Descriptions of age-specific outcomes, geographic distribution, and age and sex-statistics (tests of significance, ratios, the standard error (SE) and 95% confidence intervals) were calculated for all autochthonous disease cases per country. The SE of the log R was calculated as follows:

$$\mathrm{SE}\{\ln(R)\} = \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}$$ with a 95% CI:

$$95\%\ \mathrm{CI} = \exp\left(\ \ln(R) - 1.96 \times \mathrm{SE}\{\ln(R)\}\ \right) \text{ to } \exp\left(\ \ln(R) + 1.96 \times \mathrm{SE}\{\ln(R)\}\ \right)$$ The *z*-value was produced by using ln(R)/SE{ln(R)}; *ln* is the *natural log* scale. The significant test captured areas that were outside ±z.

Annual country incidence rates per 100,000 population by country, age group, and sex were calculated using population demographic data from Eurostat population estimates for each year of the reporting period (2010-2015).

Overall, the analysis included:

- Both probable and confirmed human WNV cases during the transmission period for the countries experiencing multiple consecutive (yearly) outbreaks
- Autochthonous, locally acquired, human WNV infections in countries reporting multiple consecutive infections during the studied period.

Notification/incidence rates were calculated as they have the potential to be valuable indicators of disease distribution in a country. The national notification rate per 100,000 population was estimated based on the number of WNV cases reported divided by the population of each studied country per year.

4.2 EUROSTAT DATA

4.2.1 Territorial Units

Human WNV infections were analysed at the Nomenclature of Territorial Units level 3 (NUTS3)/Global Administrative Unit Layers level 1 (GAUL1)[23]. The Nomenclature of Territorial Units for Statistics (NUTS) is “a hierarchical classification of administrative areas, used across the EU for statistical purposes” (Eurostat, 2017, page 1, accessed

[23] The GAUL “compiles and disseminates the most reliable spatial information on administrative units for all the countries in the world, providing a contribution to the standardization of the spatial dataset representing administrative units”. Source: https://sdi.eea.europa.eu/catalogue/eea/api/records/5f9a77a6-8f6f-4551-a158-82e138336a6f, PAGE 1.

12 September 2017). The average population size for each of the three levels (NUTS 1-3) falls within the following thresholds:

Level	Minimum	Maximum
NUTS 1	3 million	7 million
NUTS 2	800,000	3 million
NUTS 3	150,000	800,000

Source: Eurostat, 2016

NUTS 3 (using 2010) codes (the smallest territorial units made of 1,294 European regions) were extracted from Eurostat databases, the statistical office of the EU, as of 12 September 2017. For the purpose of this study, affected areas were defined as NUTS-3 level areas experiencing multiple consistently consecutive outbreaks of autochthonous human WNV cases (Table 7).

Table 7. Classification of risk areas where an arthropod-borne disease occurs/reoccurs

Risk area type	**Conditions** Environmental conditions favouring transmission	**Pathogen** Detection of pathogen in vectors and/or animals	**Transmission** Transmission to humans has occurred	**Recurrence** Seasonal recurrences of human transmission
Predisposed (*(pathogen inactive in humans and other species)*	+	-	-	-
Imperilled *(pathogen inactive in humans)*	+	+	-	-
Affected *(at least one autochthonous case)*	+	+	+	-
Endemic *(at least one recurring autochthonous case)*	+	+	+	+
Epidemic *(multiple recurring autochthonous cases)*	+	+	+	++

Note: Adapted from Domanovic and Giesecke, 2012, p1. Source: (Domanovic D, Giesecke J. How to define an area where transmission of arthropod-borne disease is occurring? Euro Surveill. 2012 May 17;17(20):20171) . Note: Risk levels require re-evaluation each season.

4.2.2 Typological data

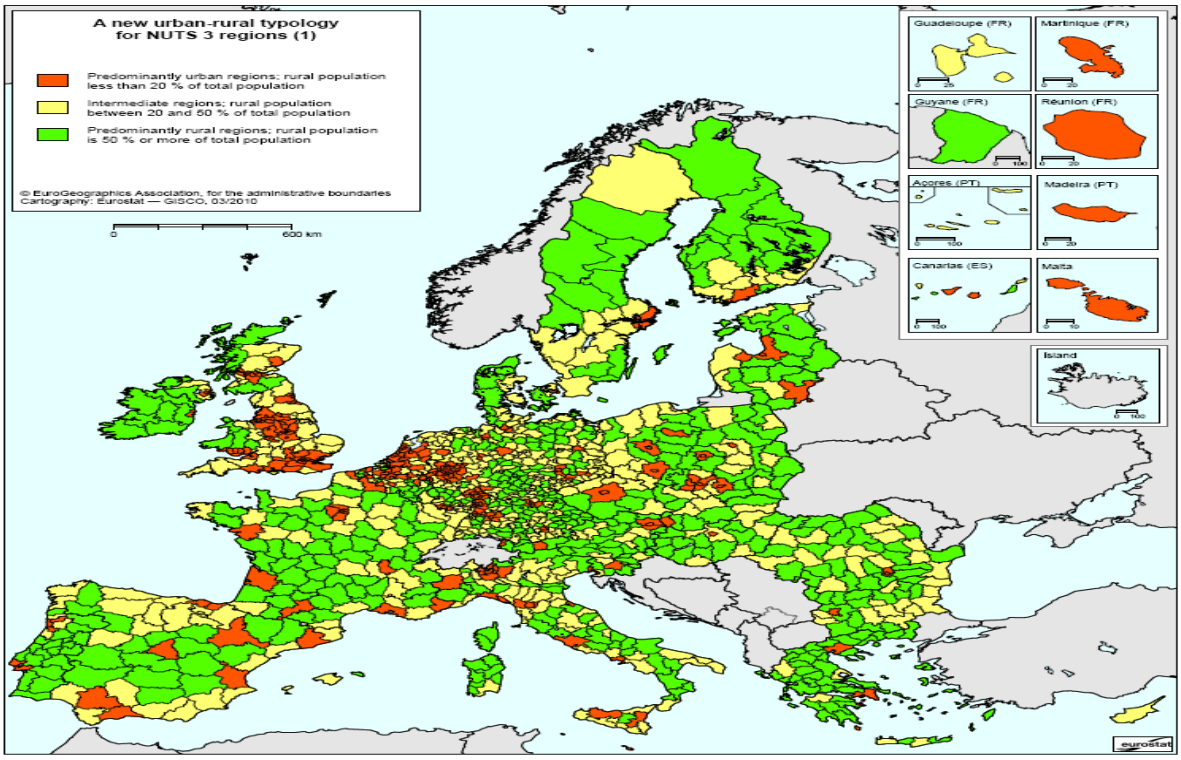
ECDC case-based data were combined with Eurostat data for 2010-2015 on rural/urban/intermediate typologies; the latter were obtained on 21 December 2015 from Eurostat's web-portal (http://ec.europa.eu/eurostat/statistics-explained/index.php/Glossary:Urban-rural_typology).

The study used Eurostat's three-step approach, which defines the share of population and classifies NUTS level 3 regions in urban, rural and/or intermediate areas. These three levels of urban/rural classification are based on the share of the population distribution living in rural areas:

- 'Predominantly rural': higher than 50% of total population
- 'Intermediate': between 20% and 50% of total population
- 'Predominantly urban': below 20% of total population (Eurostat, 2017, page 1).

The analysis compared the designate landscape typologies and the NUTS 3 areas of countries where WNV was reported present between 2010 and 2015 to estimate the most frequent typologies per affected country. Map 1 shows the landscape typologies in the EU.

Map 1 Overall urban, intermediate and rural classification in the EU



(1) This typology is based on a definition of urban and rural 1 km² grid cells. Urban grid cells fulfil two conditions: 1) a population density of at least 300 inhabitants per km² and 2) a minimum population of 5 000 inhabitants in contiguous cells above the density threshold. The other cells are considered rural. Thresholds for the typology: 50% and 20% of the regional population in rural grid cells.

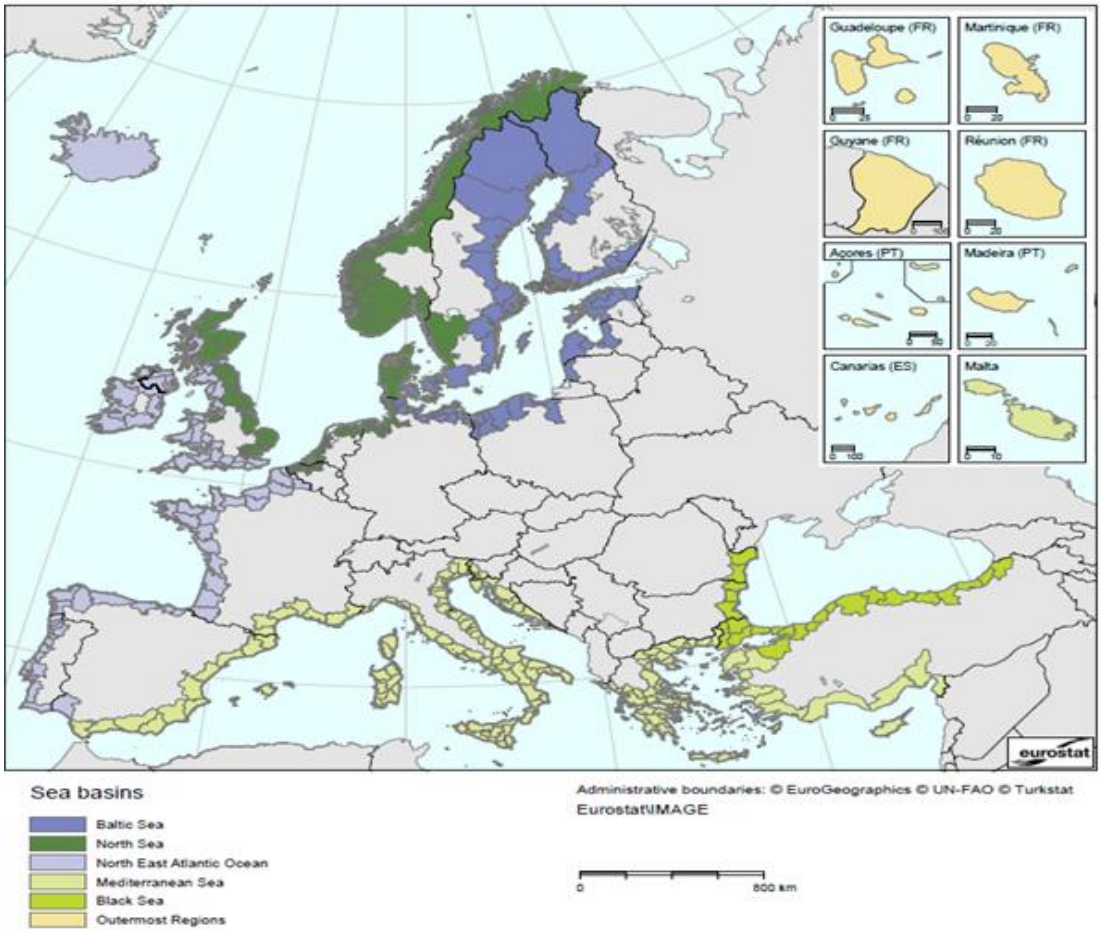
For Madeira, Açores and the French outermost regions, the population grid is not available. As a result, this typology uses the OECD classification for these regions.

4.2.3 Other regional data

In addition to the rural/urban typological data, reported WNV human cases in NUTS 3 regions were also compared against a separate Eurostat dataset which provides information on NUTS 3 approximation to coastal (or non-coastal) areas.

According to the NUTS 2010 classification, there are 1,294 NUTS 3 EU regions, with approximately 41% of the EU population living in coastal regions (Eurostat, 2017). In the EU, a coastal region is “defined as a NUTS 3 region with either a sea border or without a coastline but where more than half of the population lives within 50 kilometres (km) of the sea” (Eurostat, 2017). Map 2 provides an overview of coastal regions at NUTS 3 level.

Map 2: Coastal regions by sea basins and NUTS 3 regions



4.2.4 Population data

Population data were extracted from Eurostat's web-portal on 23 December 2015 which provides information on the population structure of the European Union (EU) per country and region.

Specifically, the study used the 'demographic balance and crude rates' (DEMO_PJAN) field and matched it to each NUTS 3 (2010) regional level with WNV presence using the data on surface area. The source of the data can be found at https://ec.europa.eu/eurostat/web/population-demography-migration-projections/data). The population, as of 1 January of each year per country, was utilised based on the average population and age structure of all EU Member States.

In order to calculate age and gender specific rates, data was divided/aggregated into six distinctive age groups (Table.8).

Table 8: Standard population per age group

Age group	Standard population
0–4	25 506 062
5–14	54 043 285
15–24	62 075 051
25–44	143 411 393
45–64	124 427 054
≥65	81 889 316
Total	491 352 161

Note: as per ECDC's methodology. Source: ECDC, 2017, p1.

The following calculations were produced:

- For reporting countries, the number of autochthonous reported/total WNV cases per country, were divided by the official Eurostat estimation of the population for that year and multiplied by 100,000. Countries with no WNV cases were omitted from the calculation of the overall European rate for WNV.
- WNV data was divided into six distinctive age groups: 0-4, 5-14, 15-24, 25-44, 45-64 and 65+ to produce rate ("r_i" is the specific rate for the age group in the equation below) for these age groups adjusted to a standard population (100,000 inhabitants). The above age divisions/groups are commonly employed in ECDC-based research.
- Age-standardised rates (ASRs) were produced to enable comparisons between countries experiencing WNV outbreaks by taking into account differences associated with certain population features such as age and gender. ASRs

were computed when the EU/EEA rate surpassed 1 per 100,000 population and was provided per 100,000 persons. The following equation for ASRs was used**:** "r_i" is the specific rate for the age group "*i*" in the studied population, and "p_i" is the population of age group "*i*" in the main population.

$$ASR = \frac{\sum_{i=1}^{6} (r_i p_i)}{\sum_{i=1}^{6} p_i}$$

4.2.5 Analytical Tools

ECDC and Eurostat data were analysed in SPPS statistic software package (Version 11.0), Microsoft Excel (version 13) and the ECDC's European Map Making Application (EMMa).

4.3 Hydroclimatic Data

The final part of this study presents the methodology of the zero-inflation mixture model based on autochthonous human WNV infections notified at EU level combined with climatological data recorded by the European Centre for Medium-Range Weather Forecasts (ECMWF) at NUTS 3 level.

Time series for air temperature, dewpoint temperature, soil temperature, volumetric soil water, wind speed (both eastward and northward), relative humidity, surface pressure and total precipitation were obtained on 20 September 2019 from the ERA-Interim Reanalysis archive of the European Centre for Medium-Range Weather Forecasts (ECMWF) and can be accessed from http://www.ecmwf.int/research/era. The Global Climate Observing System described these as Essential Climate Variables (Bojinski et al., 2014). ECMWF's ERA-Interim Reanalysis is an atmospheric reanalysis

of the global climate blending historical daily or monthly data forecasts (generated every 12 hours depending on the variable) with other more recent meteorological observations.

The archive contained longitudinal data starting from 1st January 1979 to 31st August 2019; datasets were available through the ECMWF web interface in month and/or daily frequencies. Daily data on the climatological variables for the 6-year period under investigation (2010-2015) were extracted using a Python program specifying a date range; for example, all days from 1st of January to 31 of December 2015: "date": "2015-01-01/to/2015-12-31". Full Python scripts were downloaded from the ECMWF's website and utilised accordingly to extract the data. Table 9 provides a description of each variable analysed in this study and their respective units.

Table 9. Climatic variables used in the study

Name	Units	Description
10m u-component of wind	m s-1	Eastward component of the 10m wind. It is the horizontal speed of air moving towards the east, at a height of ten metres above the surface of the Earth, in metres per second.
10m v-component of wind	m s-1	Northward component of the 10m wind. It is the horizontal speed of air moving towards the north, at a height of ten metres above the surface of the Earth, in metres per second.
2m dewpoint temperature	K *converted to degrees Celsius (°C) by subtracting 273.15.*	Temperature to which the air, at 2 metres above the surface of the Earth, would have to be cooled for saturation to occur. It is a measure of the humidity of the air. Combined with temperature and pressure, it can be used to calculate the relative humidity. 2m dew point temperature is calculated by interpolating between the lowest model level and the Earth's surface, taking account of the atmospheric conditions.
2m temperature	K *converted to degrees Celsius (°C) by subtracting 273.15.*	Temperature of air at 2m above the surface of land, sea or in-land waters. 2m temperature is calculated by interpolating between the lowest model level and the Earth's surface, taking account of the atmospheric conditions.

Total precipitation	m	Accumulated liquid and frozen water, including rain and snow, that falls to the Earth's surface. It is the sum of large-scale precipitation (that precipitation which is generated by large-scale weather patterns, such as troughs and cold fronts) and convective precipitation (generated by convection which occurs when air at lower levels in the atmosphere is warmer and less dense than the air above, so it rises). Precipitation variables do not include fog, dew or the precipitation that evaporates in the atmosphere before it lands at the surface of the Earth. This variable is accumulated from the beginning of the forecast time to the end of the forecast step. The units of precipitation are depth in metres. It is the depth the water would have if it were spread evenly over the grid box.
Volumetric soil water layer 1	m3 m-3	Volume of water in soil layer 1 (0 - 7 cm) of the ECMWF Integrated Forecasting System. The surface is at 0 cm. The volumetric soil water is associated with the soil texture (or classification), soil depth, and the underlying groundwater level.
Soil temperature level 1	K *converted to degrees Celsius (°C) by subtracting 273.15.*	Temperature of the soil in layer 1 (0 - 7 cm) of the ECMWF Integrated Forecasting System. The surface is at 0 cm. Soil temperature is set at the middle of each layer, and heat transfer is calculated at the interfaces between them. It is assumed that there is no heat transfer out of the bottom of the lowest layer.
Relative humidity	%	Relative humidity is defined with respect to saturation of the mixed phase, i.e., with respect to saturation over ice below -23C and with respect to saturation over water above 0C. In the regime in between a quadratic interpolation is applied.
Surface pressure	Pa	Pressure (force per unit area) of the atmosphere on the surface of land, sea and in-land water. It is a measure of the weight of all the air in a column vertically above the area of the Earth's surface represented at a fixed point. Surface pressure is often used in combination with temperature to calculate air density. The strong variation of pressure with altitude makes it difficult to see the low and high pressure systems over mountainous areas, so mean sea level pressure, rather than surface pressure, is normally used for this purpose. The units of this variable are Pascals (Pa). Surface pressure is often measured in hPa and sometimes is presented in the old units of millibars, mb (1 hPa = 1 mb = 100 Pa).

Source: European Centre for Medium-range Weather Forecast (ECMWF) (2011): The ERA-Interim reanalysis dataset, Copernicus Climate Change Service (C3S) (accessed 10 April 2020), available from https://www.ecmwf.int/en/forecasts/datasets/archive-datasets/reanalysis-datasets/era-interim , 2020, page 1.

The study opted for the ERA-Interim reanalysis datasets rather than other archived analyses of meteorological/seasonal forecasting systems. This is because the former provides the most comprehensive multivariable, space adjusted, and reliable record of international atmospheric presentations utilising numerical weather prediction models to address missing climatic data. As soon as data on all studied hydro-climatic variables became available by ECMWF's ERA, WNV climatic studies (Stilianakis et

al., 2016) have started utilising its datasets. Such reanalysis can produce robust homogeneous spatiotemporal data, when the latter are sparse, unaffected by methodological changes (Dee et al., 2011).

4.3.1 Statistical analysis

Based on other studies that demonstrated lag correlations between climatic factors and WNV outbreaks in human populations (Paz et al. 2013; Soverow et al. 2009) and given that West Nile Virus is a seasonal disease with *Culex* mosquitoes causing infection before the date of symptom onset, this study explored three scenarios: how the selected atmospheric variables perform and associate with disease distribution in the same month of an outbreak (lag0), one month (lag1) and two months (lag2) before the outbreak occurred. These three distinctive lags (lag 0-2) were selected based on previous empirical evidence on the short incubation period and limited movement capacity of *Culex* mosquitoes as described in Chapter 1 (CDC, 2014; Rutgers, 2011; Anderson et al., 2008; Richards et al., 2007; Reisen et al., 2006; Dohm et al., 2002). However, the lags spanned across a significant timeframe which depended on the time a WNV human case was reported. Specifically, each lag0 was relevant to the time (month) a WNV case was reported; therefore, if a case was reported in May, the study looked at May (lag0), April (lag1) and March (lag2) whereas if the infection was reported in October (lag0), September was lag1 and August lag2 respectively.

The study investigated the relationship between reported human cases of WNV at NUTS3 level and nine atmospheric factors, namely relative humidity, air temperature, dew point temperature, soil temperature, surface pressure, total precipitation, wind velocity (both eastward and northward) and volumetric soil water utilising a generalised linear model.

The mean values of these factors, during the studied 6-year reference period, were used to express the overall meteorological conditions. The values of the climatic factors within the month of the WNV human cases as well as 1 to 2 months prior to disease occurrence were utilised to discover potential associations. Individual WNV data were aggregated as 'counts' for each NUTS 3 zone.

The study utilised a zero-inflated mixture framework. Zero-inflated negative binomial (ZINB) regression is "used to count data that exhibit overdispersion and excess zeros; the data distribution combines the negative binomial distribution and the logit distribution" (Mwalili et al., 2008, p1). The model estimates the data processes (zero and counts) with two components: one logit and one binomial. The negative binomial regression (NBR) formed part of this statistical framework. it included an estimate and Pr(>|z|) set at <0.05 for all climatic parameters and theta (the true value of the parameter).

As part of the zero-inflated mixture framework utilised in this study, the climatic factors[24] (independent variables) considered were daily data (i.e., mean values of the independent variables made of the maximum (°C) and minimum air temperature (°C)) for each of the affected NUT3 areas of Greece, Hungary, Italy and Romania across the six years. Absence referred to a NUTS3 area with no reported cases and presence referred to reported WNV human infections in the same NUTS3. As such, the presence or absence (count vs. zero) of reported NUTS3 WNV human infections were employed as the dependent variables. Parameters were estimated by maximum likelihood; an intercept and a categorical variable (NUTS 3) as a regressor were used.

[24] Table 9 lists all selected climatic parameters.

The Log Likelihood value measured the fitness of the model. The lag with the highest (Log Likelihood) value across the three studied periods (lags) was deemed the best fitting model. As in any regression analysis, a residual analysis, the R package pscl was used to fit the models, similarly to other, albeit regional, WNV studies (Jackman 2015; Zeileis et al., 2008).

Climate data on a specific date, at which symptoms were recorded, can add value but more value can be achieved from knowing which climate characteristics are involved in the given month of, before and/or after, an outbreak has occurred, as explored in other studies (Paz et al. 2013; Soverow et al. 2009). It can allow useful inferences to be made on the maximum log-likelihood value and potentially provide meaningful comparisons on the fit of different coefficients to be made. Some studies have shown longer lags to be more robust than shorter lags (Rickard, Lau and Pashler, 2008) such as weekly or monthly which this study has considered and applied.

The method used to check whether collinearity occurred was by plotting the correlation matrix of all the independent variables. After plotting the correlation matrix, the pairwise correlation between all the variables were analysed. Given that most climate variables often show high collinearity, the study looked to identify if explanatory variables in the model were linearly related: very high (0.90-1.0), high (0.70-0.90), moderate (0.50-0.70) low (0.30-0.50) or negligible (0.00-0.30). These were calculated as a means of determining whether collinearity existed among those factors. The results can be found in Annex B.

CHAPTER 5- RESULTS

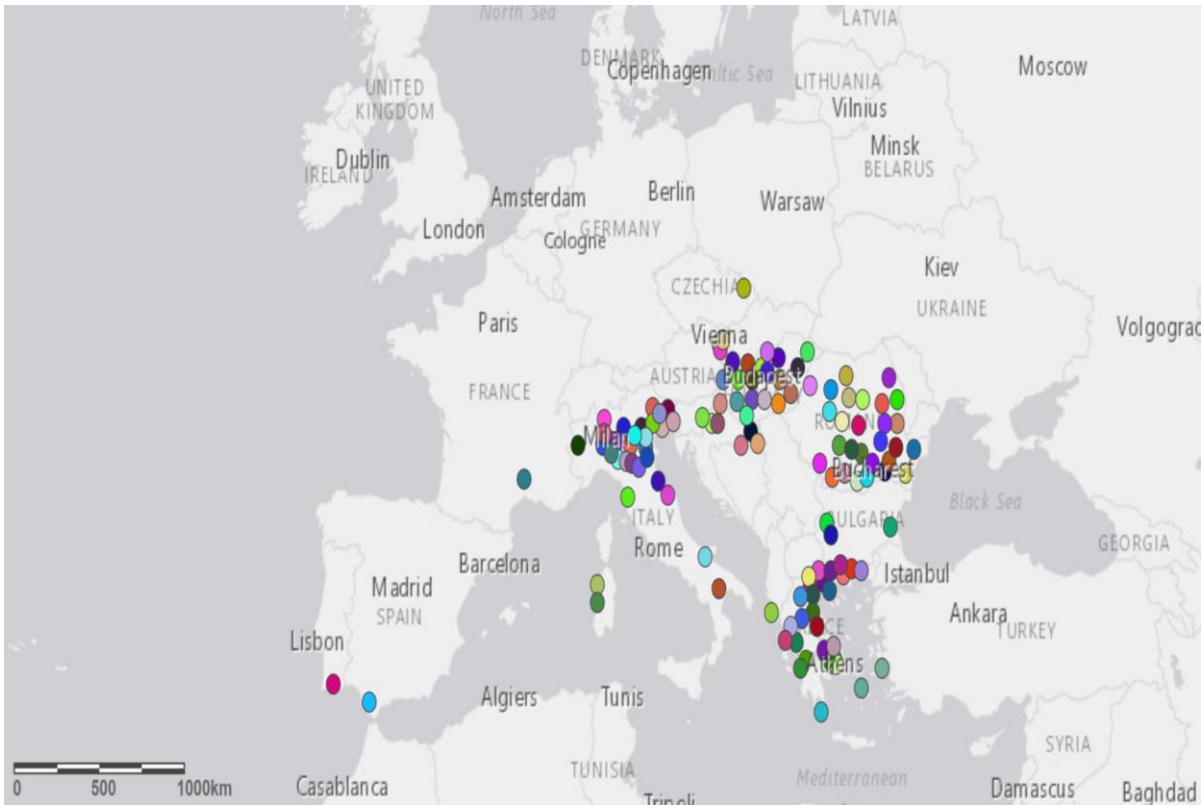
5.1 Description of WNV epidemiological trends in selected countries of the European union in human populations between 2010 and 2015.

Between 2010 and 2015, 1330 human WNV infections (average across years: (222; range: 74–340) were reported by EU Member States (Table 10-11) to ECDC's surveillance system (TESSy) at NUTS 3 (Nomenclature of Territorial Units for Statistics 3) level for the period of high mosquito activity (June–November for each respective year). Although the disease was detected in a number of countries across the EU (Map 3), locally acquired human cases (n=1210) were mainly reported in the south-eastern parts of the EU, namely Greece, Hungary, Italy and Romania (Map 4) during the transmission season (June to November).

5.1.1 Number and geospatial distribution of human WNV cases

Between 2010 and 2015, Greece had the highest number (623 (57%)) of WNV cases in humans between June and October compared to other reporting countries, affecting 11 of its 13 regions followed by Italy, with 323 human cases (19% of the EU's total for that period in 8 of the 20 regions), Romania (14%) and Hungary (9%). Annual seasonal outbreaks were detected between June and October in human populations across all these countries over the study period and continued until 2015, except for Greece, which saw a rapid decrease in cases in 2014 (down to 15 from 262 in 2010) and no reported cases in 2015.

Map 3: Distribution of all autochthonous human WNV infections in countries of the European Union, 2010-2015



Note: the map was produced using ECDC's EMMa. Colours represent the NUTS 3 regions reporting human WNV infections.

In 2010 (Table 10-11 and Maps 4-9), 99% of 247 human cases of WNV were locally acquired, reported across many EU countries (average: 85, the high across 2010-2015): overall, the following EU Member States reported WNV human transmission: Austria, Greece, Hungary, Italy, Romania and Spain. This was the second largest human WNV epidemic in the EU after the unprecedented 1996 outbreak in south-eastern Romania (Tsai et al., 1998). In Greece, a large outbreak was observed during July–October, primarily affecting its northern parts, namely 7 areas of Central Macedonia, with 262 cases submitted to the ECDC. Romania reported 57 cases, mostly in its south-eastern regions of Constanta and Ialomita. Both country outbreaks peaked towards the end of August, gradually subsiding in September. This indicates that certain conditions in that year created a favourable environment for the circulation

of WNV in both Greece and Romania. Certain climatic factors such as temperature, humidity and others will be analysed at a latter part for this study as a means of better understanding these outbreaks.

In 2011 (Table 10-11, Maps 4-9) all146 cases reported in the EU were autochthonous infections. WNV transmission was detected in the following EU Member States: cases from Greece (n=99; a 31% decrease from 2010), Italy (n=32), Hungary (n=4) and Romania (n=11). WNV was reported in many and different regions across the four countries: central Macedonia was one of the main epicentres for Greece, but Attica (southern Greece) reported the highest number (n=30) of infections in a single prefecture. Bucharest (n=6) was the epicentre for Romania whereas numbers were equally distributed between central and eastern Hungary. Almost half of the reported cases for Italy in 2011, came from a single northern region (Treviso, n=6).

In 2012 (Table 10-11, Maps 4-9), the number of WNV human cases in the selected EU countries was lower than 2011; 2013 data showed a similar trend. 97% of 266 cases were autochthonous; 161 cases came from Greece and 73 from Italy. Greece's north-eastern (Xanthi prefecture=44) and Attica prefectures (n=45) reported the highest numbers in a single area; the highest cumulative numbers in Italy were reported in the Veneto region of northern Italy and in Bucharest (n=6) for Romania. Hungarian WNV cases were spread equally across its regions.

In 2013 (Table 10-11, Maps 4-9), of a total of 293 cases 271 (97.2%) were locally acquired reported cases in EU countries (the second highest rate for the period between 2010 and 2015), with four EU Members States reporting continuous disease presence: Greece (n=86) and Italy (n=126), continued to report the highest numbers of cases. Attica prefecture (n=35) in Greece, Budapest (n=6) in Hungary, Modena

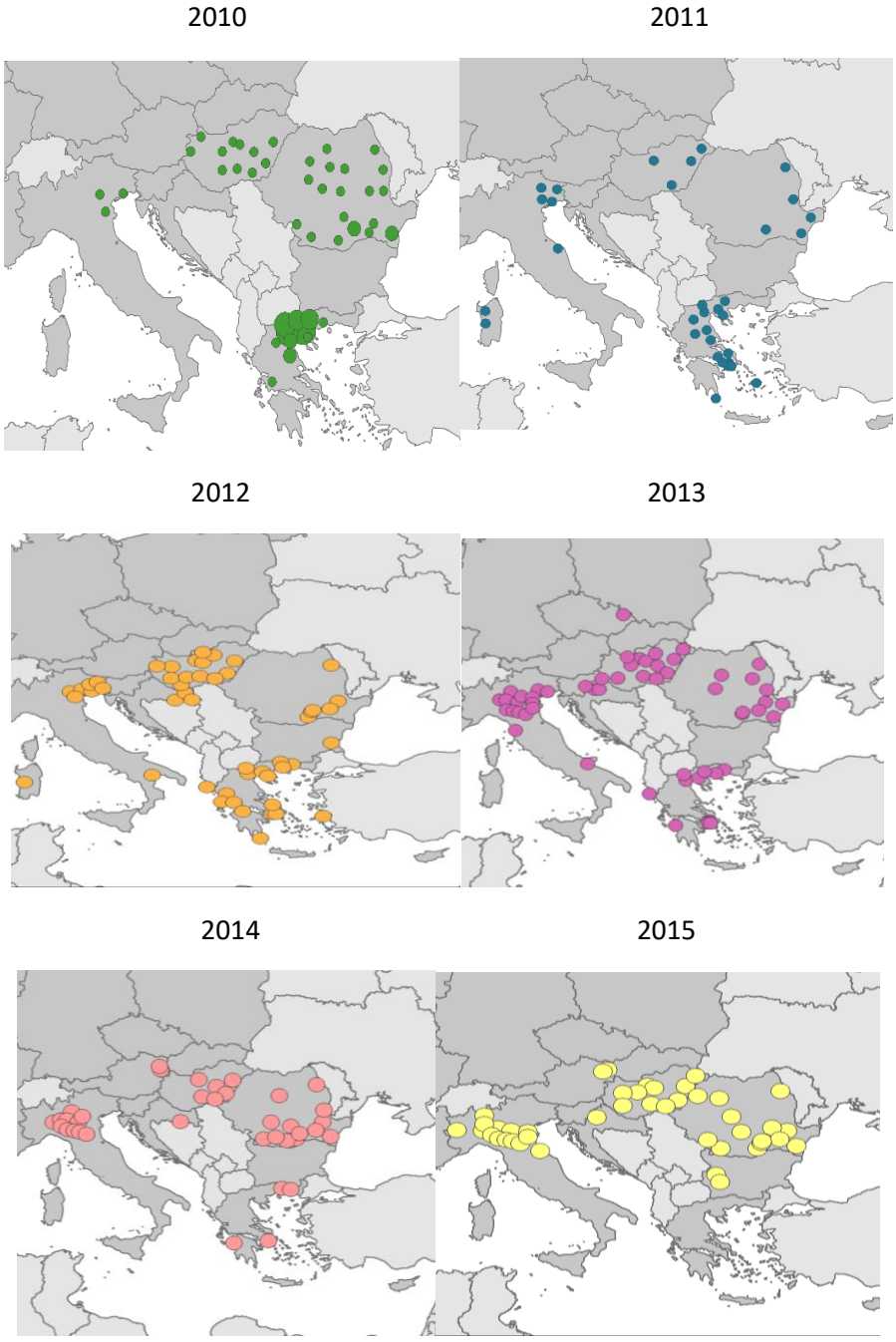
province (n=16) in Italy and Braila (n=4) in Romania reported the highest numbers in a single NUT3 area even though locally acquired cases were spread equally across their respective regions. Other EU Member States such as Croatia (n=16), Czech Republic (n=1) and Slovenia (n=1) reported human WNV cases but excluded from the analysis as they did not meet the inclusion criteria of this study (Map 3).

In 2014 (Table10-11, Maps 4-9), 72 (93.6%) of the 75 WNV cases in the EU were locally acquired, 86% of which were confirmed, with a yearly average of 19; these were reported by the following countries: Greece (n=15), Hungary (n=10), Italy (n=24) and Romania (n=23). The highest number of cases per country in a single unit were reported in Greece's northern Rodopi prefecture (n=8), the province of Pavia (n=5) in northern Italy, the southern Romanian district of Olt (n=6) and Hungary's capital Budapest (n=4). Following 3 years of consecutive outbreaks, Greece only reported 15 cases in 2014 in only four (two northern and two southern) regions: Attica, Ileia, Rodopi and Xanthi prefectures. Most WNV cases in Italy came from its northern regions such as Bologna (n=4) and Pavia. Romania's southeast and central regions continued to be affected, similarly to previous years and reported both imported and autochthonous human cases.

In 2015 (Table 10-11, Maps 4-9), there were 111 (90.6%) autochthonous cases in the selected EU countries, with a yearly average of 27. The highest numbers of cases (n=61) were recorded in Italy; infections were reported in highly populated Italian regions, such as Milano, a region with no history of local human WNV transmission. The levels of endemicity In Hungary (n=18 in 2015) and Romania (n=32 in 2015) were similar to 2014, affecting their capital cities and densely populated areas, Budapest (n=7) and Bucharest (n=5); however, the vast majority of infections were reported in other regions. Similar to 2013, 20% of cases in Italy came from the Modena province

(n=12) in northern Italy with 39% increase compared to 2014. Following a series of outbreaks in earlier years, Greece reported no cases for the first year since 2010 (n=262).

Maps 4-9: Distribution of (locally acquired) human WNV infections in EU countries experiencing consecutive outbreaks between 2010-2015



Note: maps were produced using ECDC's EMMa. In the 2010 map, larger points indicate cases between 10-160. Light grey: non-EU countries.

5.1.2 Incidence rate per 100,000 person-years in affected EU Member States, 2010-2015

Table 10 shows that Greece had the highest incidence rate of 8.15 cases per 100 000 people at risk of developing the disease in 2010 when it experienced several outbreaks in Central Macedonia and the second highest of 2.43 cases in 2012 across all studied years and countries. Hungary had the lowest (0.12) incidence rate of population at risk of developing WNV 2011 (lowest across all countries and years), which also corresponds with the low notification rate and number of cases. Greece did not report any cases in 2015. Italy had the largest incidence rate of 0.71 cases per 100,000 persons in 2012 and 2013 respectively and Romania of 0.56 cases in 2010. Romania's incidence rate decreased in subsequent years but remained relatively stable across years whereas Greece's rate saw a noticeable decrease in 2014. Hungary and Italy's incidence proportion per 100,000 population oscillated over time.

Table 10. Annual incidence per 100,000 population of West Nile virus, by year and country - Selected EU Countries*, 2010-2015

Country	Year	Total Population at risk*	No of Regions at Risk*	Autochthonous cases	Incidence rate per 100,000 person-years
Romania					
	2010	10210355	20	57	0.56
	2011	4089105	5	11	0.27
	2012	4440038	7	15	0.34
	2013	6021371	10	24	0.40
	2014	8078134	16	23	0.28
	2015	8258635	14	32	0.39
Hungary					
	2010	6649675	10	18	0.27
	2011	3252489	4	4	0.12
	2012	6491849	12	17	0.26
	2013	5694163	9	35	0.61
	2014	5177566	7	10	0.19
	2015	4198667	6	18	0.43
Italy					
	2010	1706167	2	7.00	0.41
	2011	2783600	6	32	1.15

	2012	3933358	8	73	1.86
	2013	10873780	16	126	1.16
	2014	6555508	11	24	0.37
	2015	9281088	15	61	0.66
Greece					
	2010	3213508	12	262	8.15
	2011	6931432	15	99	1.43
	2012	6671848	14	161	2.41
	2013	6327889	9	86	1.36
	2014	4665506	4	15	0.32
	2015	0	0	0	0

* Greece, Hungary Italy and Romania.

5.1.3 WNV notification rate in affected EU Member States, 2010-2015

Table 11 shows that both the highest and lowest notification rates across the six years period were recorded in 2010. Greece had the highest notification rate for locally acquired cases of 2.36 cases per 100,000 population and only 0.01 cases were notified by Italy the lowest for that year and across all studied years. The second highest notification rate across the EU/EEA was also in Greece in 2012 (1.45 cases per 100,000 population), with Italy (2014) and Hungary (2011) holding the second lowest rate of 0.04 cases per 100,000 population across all years respectively. Greece has the highest notification rate in EU/EEA from 2010 to 2014 and Hungary for 2015. Romania's notification rates remained relatively stable across the study period (especially over the 2013-2015 period) whereas Greece's rates decreased over time and Hungary and Italy's fluctuated.

Overall, the 2015 notification rate of WNV in humans in the EU/EEA was higher than 2014 but not as high as in 2013 and 2012. The 2014 EU notification rate for autochthonous cases was 0.01 cases per 100,000 inhabitants, lower than the previous year (0.33 cases). It is worth noting that country-specific surveillance systems differ therefore notification rates will vary accordingly which makes country to country comparisons challenging.

Table 11: Notification Rate and Age Standardised Rate per selected EU country, 2010-2015

5.1.4 West Nile Virus Age Standardised Rate (N/100000) per age groups in affected EU Member States, 2010-2015

Annual country data were divided into six distinctive age groups: 0-4, 5-14, 15-24, 25-44, 45-64 and 65+ to produce rates for all ages adjusted to a standard population (100,000 inhabitants). The highest ASR in the affected countries was in 2010 (0.08 cases per 100,000 inhabitants) (Table 11) and lowest in 2014 (0.02 cases). Table 12 shows, that apart from Hungary, the highest ASR per country was in those aged 65 and over.

Table 12. West Nile Virus Age Standardised Rate (N/100000) per age group for Hungary, Greece, Italy and Romania, 2010-2015

Country	0-4	5-14	15-24	25-44	45-64	65+
Greece	0.36	0.28	0.32	0.54	1.82	**8.72**
Hungary	0.20	0.00	0.16	0.20	**0.23**	0.18
Italy	0.00	0.00	0.00	0.01	0.00	**0.04**
Romania	0.00	0.05	0.16	0.24	0.37	**0.55**

ASR was high in Hungary for individuals in the second oldest group (45-64s) with 0.23 cases per 100,000 people which may have influenced this result (Table 12). There were no previous studies identified to make direct comparisons to these results. Additionally, some ASR variations were observed between and amongst countries. For example, Hungary was the only country that did not produce its highest ASR in the over 65s age group, Romania recorded high ASR in those 44-64 groups, Italy reported infections in those 25-44 years of age but mostly in those 65 and over and Greece primarily in individuals 65 and over. A statistically significant difference

between those 65 and over compared to the remaining age groups was found across all four countries with WNV more likely to occur in those over 65 years of age apart from Hungary (0.3 CI: 0.2 to 0.5) (Table 13).

Table 13. WNV autochthonous cases per country: ratio of acquiring a WNV infection for individuals 65 and over compared to those aged 0-64, 2010-2015

	Greece	Italy	Hungary	Romania
Ratio	7.013	5.6683	0.3746	1.961
95 % CI:	5.9442 to 8.2739	4.4839 to 7.1655	0.2747 to 0.5108	1.2976 to 2.9636
z statistic	23.089	14.507	6.206	3.197
Significance level	P < 0.0001	P < 0.0001	P < 0.0001	P = 0.0014

Hungary

For Hungary (Table 11, Table14), its highest ASR was 0.35 cases per 100,000 inhabitants in 2013 and lowest (0.04) in 2011 across all years. ASRs for 2010, 2012 and 2015 were similar, ranging from 0.17 to 0.18 per 100,000 inhabitants. ASRs for most age groups, apart from 5-14 years which was 0, were analogous, ranging from 0.16 per 100,000 people for those aged 15-24 to 0.23 for 45-64s, which was the highest for the country across all age groups (Table 12).

Greece

For Greece, (Table 11; Table 14), its highest ASR across all years was 2.15 cases per 100,000 inhabitants in 2010 and lowest (0.12) in 2014. ASRs for 2011 and 2013 were similar, ranging from 0.70 (2013) to 0.80 (2011) cases per 100,000 inhabitants. Greece had its second highest ASR across all years in 2012 (1.32 per 100,000 inhabitants) which corresponds with the high infection rate for that year. As regards the individual age groups, Greece had the highest ASR (8.72 cases per 100,000 inhabitants) (Table

12) for those over 65 followed by 1.82 cases per 100,000 people for individuals between 45-65 years. All other age groups demonstrated similar rates ranging from 0.28 cases (5-15) to 0.54 cases (25-44). The ratio for those 65 and over was 7.0 (CI: 5.9 to 8.2) (Table 13).

Italy

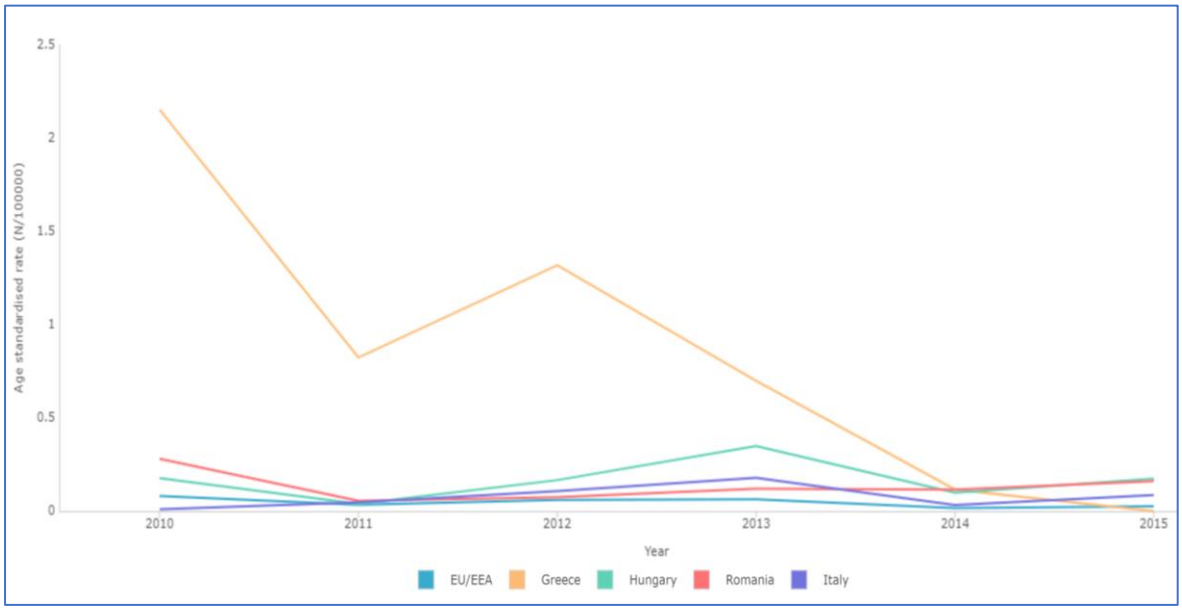
For Italy (Table 11; Table 14), its highest ASR across all years was 0.18 cases per 100,000 people in 2013 and lowest (0.01) in 2010. ASRs for 2012 and 2015 were similarly low, ranging from 0.09 (2015) to 0.11 cases (2012) per 100,000 inhabitants. Alike Greece, those 65 and over had the highest ASR across all other age groups (0.04 per 100,000 inhabitants) (Table 13). Italy only had one other age group, those between 25-44, with ASR 0.01 cases per 100,000 people. For all other age groups, namely 0-4, 5-14, 15-24 and 45-64, the rate that its population would develop WNV, if it had a standard age structure, was zero; this suggests that its, at risk, populations mainly fall into 2 main age groups: 25-44 and those 65 and over. The ratio for those 65 and over was 5.6 (CI: 4.4 to 7.1) (Table 13).

Romania

For Romania (Table 11; Table 14), its highest ASR across all years was 0.28 cases per 100,000 people in 2010 and lowest (0.06 cases) the following year. For all other years, the age standardised rate remained relatively stable with small fluctuations ranging from 0.07 (2012) to 0.16 (2015) cases per 100,000 inhabitants. Similar to Greece and Italy, those 65 and over had the highest ASR across all other age groups (0.55 cases per 100,000 inhabitants) (Table 12); only those between 0-4 years had zero age standardised rate which makes it the only unaffected group. For all other age groups (Table 12), namely 5-14, 15-24 and 45-64s, the ASR become gradually higher

for older age groups, ranging from 0.05 for those between 5 and 14 years of age to 0.37 cases per 100,000 people for individuals in the second oldest group (45-64s). The ratio for those 65 and over was 1.9 (1.9 CI:1.2 to 2.9) (Table 13).

Table 14: Age Standardised Rate (N/100000) of autochthonous human WNV reported cases in Hungary, Greece, Italy and Romania per year (2010-1015)



5.1.5 West Nile Virus (locally acquired cases) gender distribution in affected EU Member States, 2010-2015

Table 15 presents the median age per gender in each of the affected EU Member State. Table 16 shows that over 59.4% of reported human WNV infections recorded across the four countries were male. In 2010, which recorded the highest number of infections, over 66.4% of reported cases were male; a similar trend was observed in the following years were males held the highest percentage of infections compared to females: 53.8% in 2011, 55.9% in 2012, 58% in 2013, 69% in 2014 and 53.4% in 2015. 2015 recorded the lowest rate of WNV infections for men compared to women across the years and there may be a link between Greece's absence of reported cases. Italy's

high proportion of cases in men in 2014 (75%) may have contributed to the highest percentage of male infections across the years and countries.

Hungary

57% of the autochthonous reported cases were men who had an increased ratio of 1.7 (CI:1.1 to 2.5) compared to women to develop WN disease across the years. For men, the highest age median was 61 (2013) years (Table 15). The highest median age for females was 70 (2011) and lowest 30 in 2010 (Table 15). Hungary had the lowest median age for both females (49) and males (56) across all studied countries.

Italy

Men had a significantly higher ratio (4.7 CI: 3.7 to 5.9) of contracting WNV than women with a p-value as low as $P < 0.0001$ (Table 13). 71% of the 323 cases reported over the 6 years were male over 65 years. For men, the highest age median was 73 (2011) years and lowest 56 in 2010. The highest median age for females was 86 (2011) and lowest 63 in 2015 (Table 15).

Greece

Over 59% of the 623 autochthonous cases between 2010 and 2014 were male over 65 years (Table 16). The highest median age for females was 76 (2014) and lowest 68 in 2012 (Table 15). For men, the highest age median was 81 (2014) years and lowest 65 in 2011. Greece had the highest median age for both females (72) and males (73) across all countries with an increased ratio of 4 (CI:3.4 to 4.8) for men compared to women (with a p-value as low as $P < 0.0001$) to contract WNV infections during the studied period (Table 17).

Romania

The ratio for a WNV infection was greater for men at 1.7 (CI:1.29 to 2.4) compared to women (with a p-value considerably less than 0.05) (Table 17) during 2010-15. Around 60% of the 162 cases across the 6-year period were male over 65 years (Table 16). The highest median age for females was 67.5 (2012) and lowest 55 in both 2013 and 2014. For men, the highest age median was 81 (2011) years and lowest 42 in 2014 (Table 15).

Table 15. West Nile Virus (locally acquired cases) median age by gender in affected EU Member States, 2010-2015

	2010		2011		2012		2013		2014		2015		Age Median		Age Median
Country	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**Total**
Hungary	30	53	70	52	57	57	38	61	66	48	59	60	49	56	**55**
Greece	72	73	72	66	68	68	71	75	76	81	n/a	n/a	72	73	**71**
Italy	0	56	86	73	64	69	69	71	67	69	63	68	69	69	**69**
Romania	60	56	62	81	68	60	55	49	55	42	59	60	60	57	**59**
Total	**45**	**56**	**71**	**69**	**66**	**64**	**62**	**66**	**66.3**	**58**	**59**	**60**	**64**	**63**	**64**

Note: n/a means that that there were no reported cases for that year. In 2010, Italy's reported cases were all male.

Table 16. Percentage (%) of men locally acquiring WNV infections in Hungary, Greece, Italy and Romania, 2010-2015

	EU/EEA	Hungary	Greece	Italy	Romania	(%)
2010	55.6%	61.1%	52.3%	100%	63.2%	66.4%
2011	61.6%	25%	62.6%	65.6%	54.5%	53.8%
2012	58.4%	52.9%	57.1%	64.4%	46.7%	55.9%
2013	59.4%	60%	51.2%	61.1%	58.3%	58%
2014	68%	70%	73.3%	75%	60.9%	69.4%
2015	73.8%	72.2%	n/a	60.3%	59.4%	53.14%
Total	63%	57%	59%	71%	57%	59.4%

Table 17. WNV autochthonous cases per country: ratio of men acquiring WNV compared to women, 2010-2015

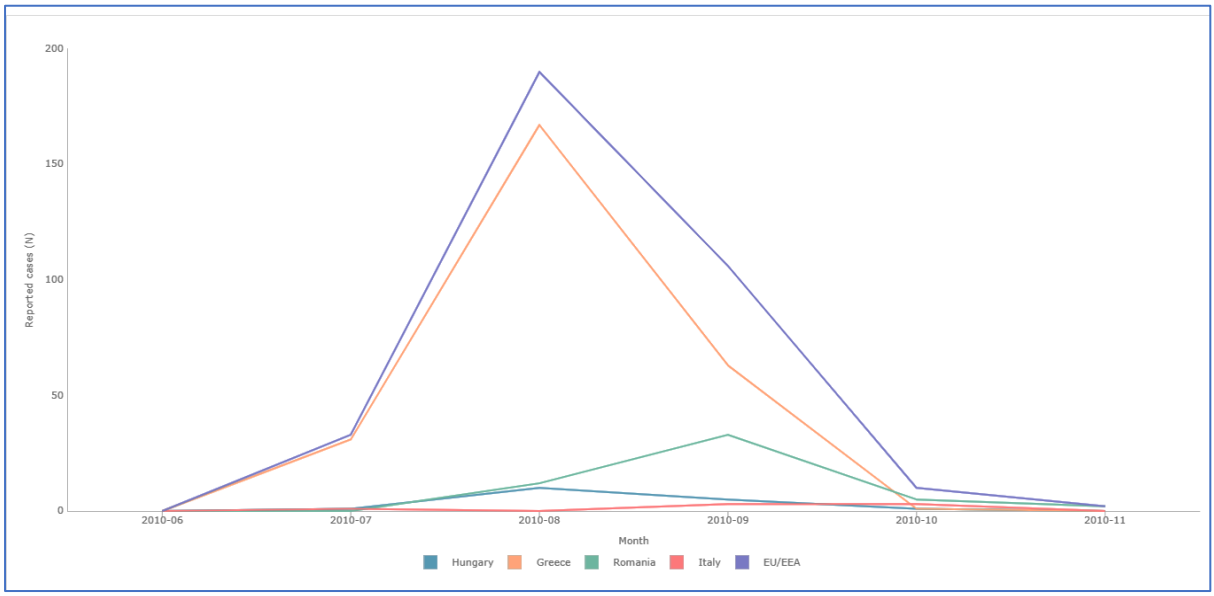
	Greece	Italy	Hungary	Romania
Ratio	4.0875	4.7408	1.738	1.7611
95 % CI:	3.4179 to 4.8884	3.7659 to 5.9679	1.1789 to 2.5622	1.2913 to 2.4018
z statistic	15.423	13.250	2.791	3.575
Significance level	P < 0.0001	P < 0.0001	P = 0.0052	P = 0.0004

5.1.6 West Nile virus transmission season in affected EU Member States, 2010-2015

The WNV transmission season for human populations across the six years period covered the months between June and November with marginal variations as regards the week of the month of onset and disease peak off, usually peaking between mid-August and mid-September. WNV disease circulation was found to be present during the period commonly associated with *Culex* activity and ranged from mid-June to mid-November. The following tables (18-23) present how autochthonous cases were distributed per country during the transmission period. The reported cases are not age standardised as the aim here was to show the movement of the virus over the months per country rather than compare WNV's monthly transmission between countries.

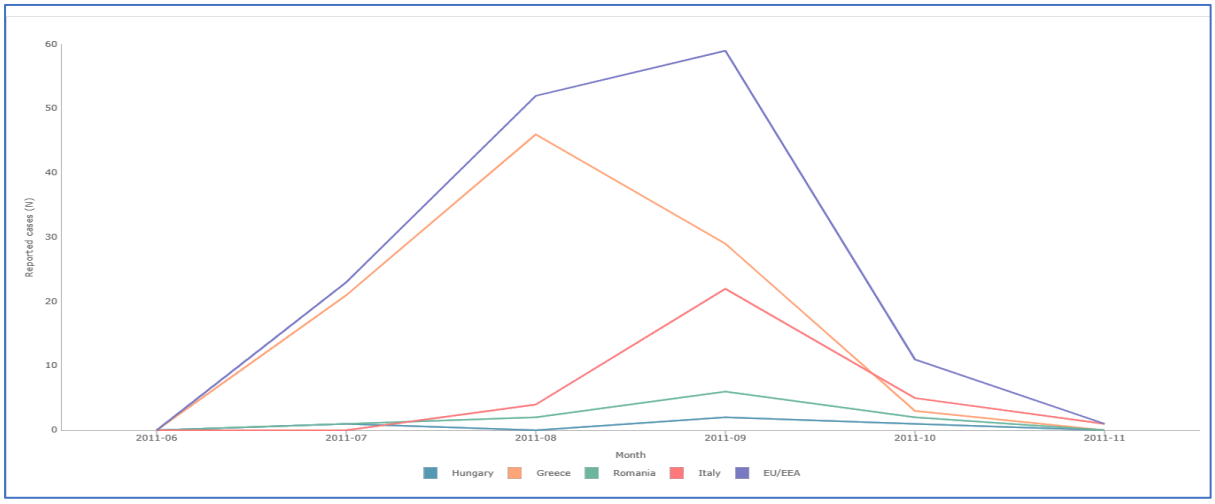
In 2010 (Table 18), reported disease peaked in mid-August (weeks 33-34) for Greece and Romania (as high as 65% of reported cases) and mid-September (week 38) for Italy and Hungary. For Greece and Hungary, cases started peaking from week 29 (early July) and gradually dropping around weeks 41-42 (early October); the latter was also the case for Italy and Romania.

Table 18: West Nile virus (case) transmission season (June-November) in affected EU Member States, 2010



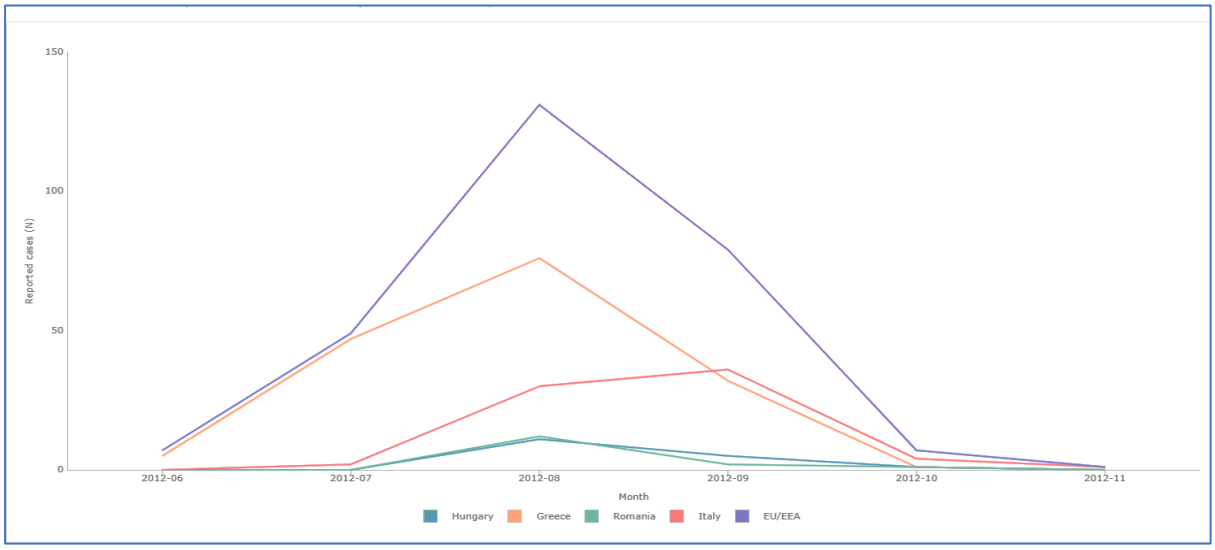
In 2011 (Table 19), the onset of WNV transmission in Europe started in Greece, earlier than previous years in early-mid June (weeks 24-25) settling in late October –early November (week 44-45). For Italy, Romania and Hungary cases peaked in early September (week 37) although disease was present throughout all previous months. Unlike 2010, disease transmission dropped off less steeply during weeks 41 and 48.

Table 19: Human WNV cases during the transmission season (June-November) in affected EU Member States, 2011



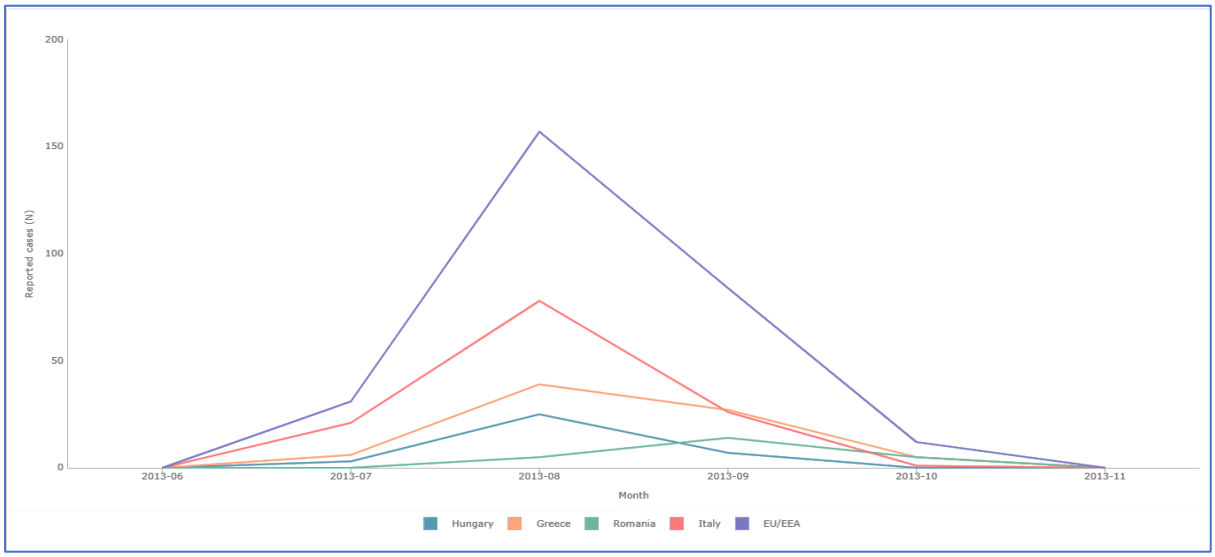
In 2012 (Table 20), WNV human disease circulation peaked in August for Greece, Romania and Hungary and, alike previous years, in September for Italy although a rapid increase in cases started occurring from week 32 (early August) onwards. On the other hand, Greece reported ¼ of its total cases in early-July (week 28). Unlike 2011, human WNV transmission decreased more sharply from October onwards during weeks 41 and 48.

Table 20: Human WNV cases during the transmission season (June-November) in affected EU Member States, 2012



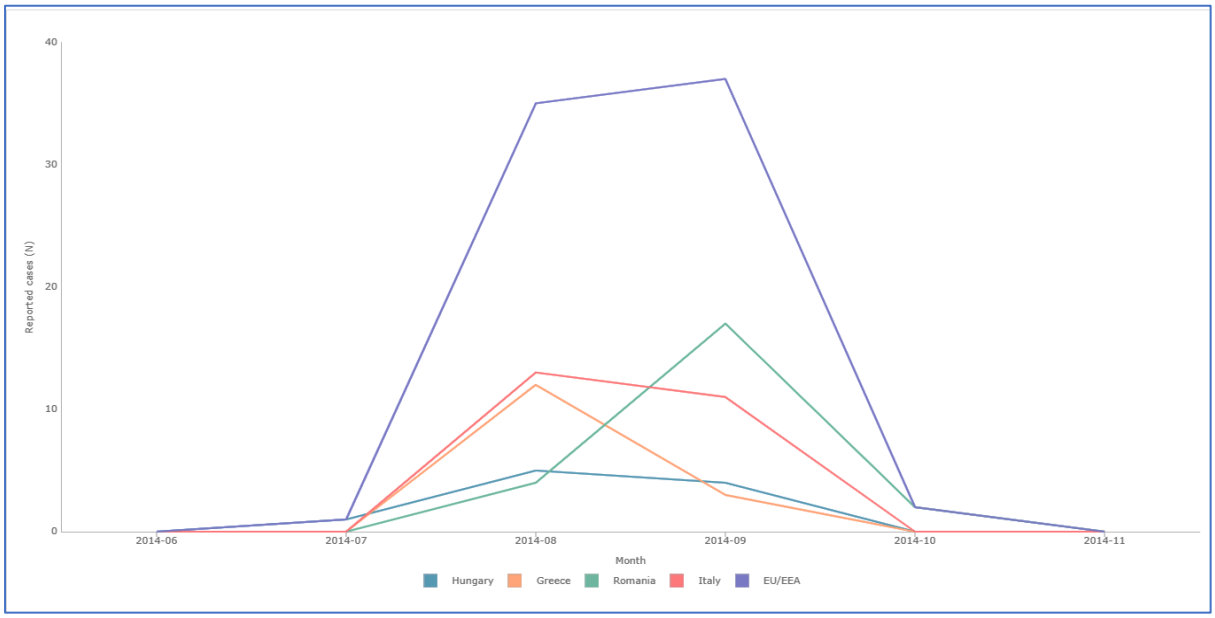
In 2013 (Table 21), WNV human disease transmission peaked in August for Greece, Hungary and Italy. Although Greece had almost half of number of the cases compared to Italy, the decrease in cases was less gradual in weeks 37-39 (September) as opposed to Italy. Similar to 2011, Romania reported most of its cases in September (weeks 37-39) with a gradual increase in cases occurring from week 32 (early August) onwards. Except for 2010, Hungary had same trends in terms of peak (early August) and decrease (early October) of cases as previous years.

Table 21: Human WNV cases during the transmission season (June-November) in affected EU Member States, 2013



In 2014 (Table 22), WNV human disease circulation increased in August for Greece, Hungary and Italy although cases were detected as early as June with number steadily increasing from weeks 29-30 (early mid-July) and gradually dropping around weeks 41-42 (early October); Hungary and Italy's transmission rates peaked again in September but were not as high as in August. Greek cases started dropping off in September (weeks 37-38) in a rapid manner whereas Italian disease transmission was more prominent compared to Greece and the decrease less sharp during these weeks. Romania reported most of its cases in September (weeks 37-39), but transmission decreased less sharply compared to other countries from October onwards during weeks 41 and 48. The 2014 seasonal transmission resembled 2010 seasonal case distribution patterns.

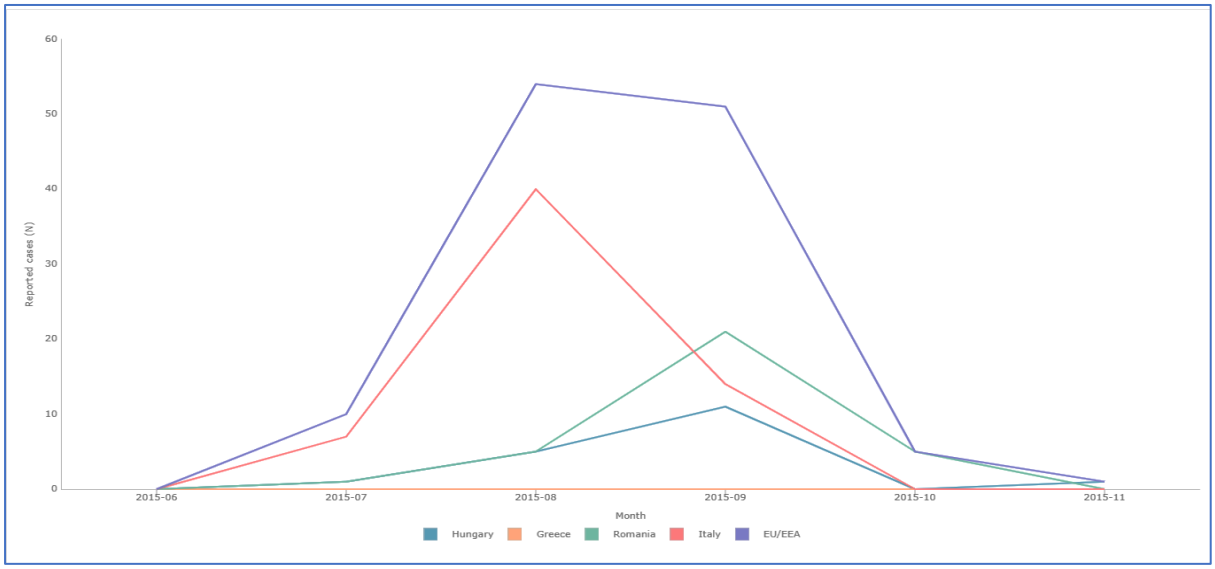
Table 22: WNV cases during the transmission season (June-November) in affected EU Member States, 2014



In 2015 (Table 23), WNV human disease transmission peaked in August for Italy but declined sharply in October. The increase in numbers commenced in weeks 29-30 (early mid-July) with early cases being detected as early as June. WNV circulation increased significantly in September for Hungary and Romania with Italian disease transmission declining around the same period, although cases in Hungary steadily decreased around weeks 41-42 (early October).

Romania's decrease was less steep, but more gradual, spanning across late October-early November (weeks 44-45). Following 2014, were a significant drop, compared to other years, in cases was recorded in Greece, there was no WNV circulation reported in 2015.

Table 23: WNV cases during the transmission season (June-November) in affected EU Member States, 2015



5.2 Environmental factors per year and country: proximity to coastal areas and landscape typologies

Tables 18, 26, 28 and 30 show that, although human West Nile disease transmission between 2010 and 2015 occurred in both coastal and non-coastal areas - with the exception of Hungary - as much as 63.7% of cases across the four studied countries, namely Hungary, Greece, Italy and Romania, were detected in non-coastal areas. Greece had no infections reported in 2015 and a relatively higher infection rate in coastal regions across the 4 years which may have influenced the result. 14 of Hungary's 16 counties reported autochthonous infections in 2012 and with the lowest number of regions affected in 2011 (n=4) whereas Greece reported the highest number in 2010-11 and lowest in 2014 (8 out of 54 prefectures[25]). Italy had 15 of its regions affected in 2013 and only two in 2010, the lowest number of regions affected during 2010-2015 across all countries. Almost half of Romania's counties were

[25] Each country uses a different terminology to describe its regional divisions. For example, Greece uses prefectures whereas Hungary uses counties.

affected in 2010 (19 out 41) but only 8 in 2012 which was the highest year for neighbouring Hungary (Tables 24-25, 27, 29 and 31). Italy (Table 29) had more cases being reported in intermediate zones across the years, however there were more rural NUTS3 locations than intermediate reporting disease presence. This demonstrates that the total number of cases does not always align with the volume of NUTS3 affected areas as a single typology i.e., urban, may have more cases than multiple rural areas with less cases.

The following section provides a brief analysis of the reported WNV cases per typology.

- **Rural regions** were commonly associated with disease transmission across the years and selected EU countries:
    - in Hungary (53.3%), Greece (81.8%) they were the leading landscape typology, and
    - the second most affected areas in Italy (30.3%) and Romania (39%) where human WNV cases circulation occurred.
- **Intermediate zones** observed the highest rates in Italy (62.5%) and more increased circulation rates in Romania (42.3%) compared to rural geographies (39%).
- **Urban areas** were the second highest affected for Greece (16.5%) and Hungary (23.8%) but the least associated with disease occurrence in Italy (7.3%) and Romania (18.4%).

Table 24. Percentage (%) of autochthonous WNV human cases in landscape typologies in Hungary, Greece, Italy and Romania, 2010-2015

Country	Intermediate	Urban	Rural
Hungary	22.9%	23.8%	53.3%
Greece	2.0%	16.5%	81.8%
Italy	62.5%	30.3%	7.3%
Romania	42.3%	18.4%	39.2%

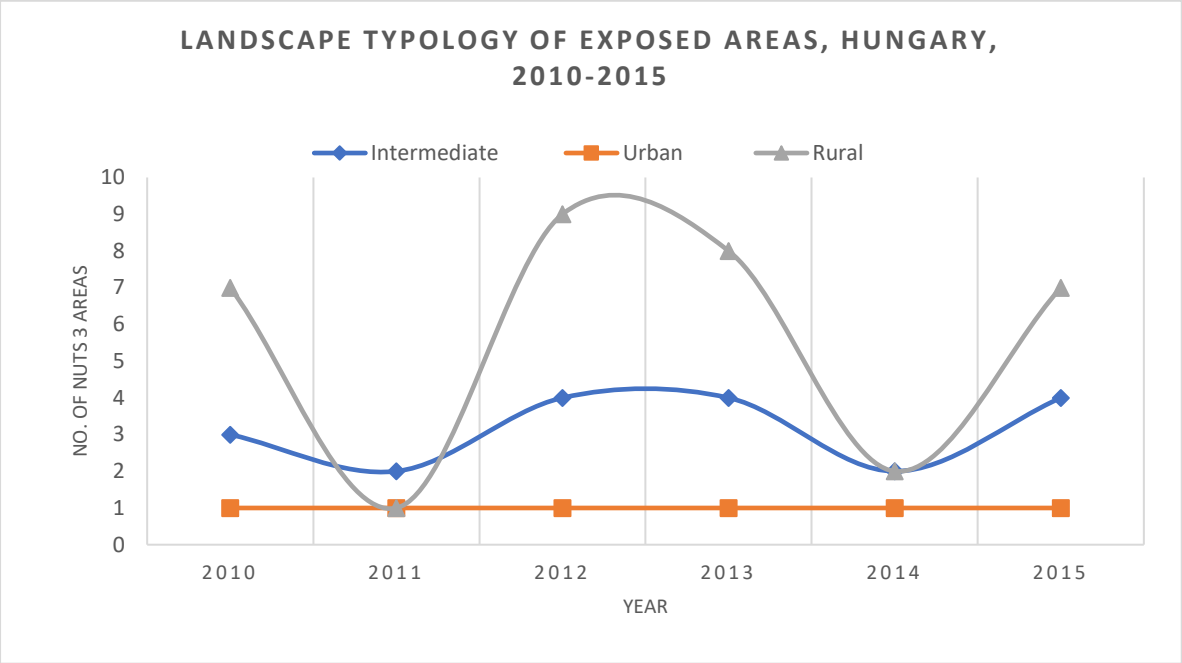
Note: Greece did not report any cases in 2015

Hungary

Hungary has no coastlines therefore all reported, locally acquired, cases in Hungary over the six-year period (2010-2015) were approximating non-coastal areas including lakes. Those living in rural areas (Table 25) were most affected (53.3% of reported cases), 23.8% in urban and 22.9% in intermediate areas; human WNV was the least prevalent across the 6-year period in one rural area (Szabolcs-Szatmár-Bereg county) in 2011 followed by only three rural regions 2014 (Bekes, Bacs-Kiskun and Jasz-Nagykun-Szolnok counties).

2013-2015 recorded higher numbers of infections in urban compared to intermediate areas which resulted in higher overall urban infection rates**.** These results suggest that rural areas were human WNV hotspots for Hungary across the studied period and intermediate areas had the lowest infection rate. In terms of the number of NUTS 3 areas, the vast majority were rural areas except for 2011 where more intermediate zones were affected by WNV (Table 25).

Table 25: Landscape typology (urban, Intermediate or rural) per number of WNV exposed areas (NUTS 3), Hungary, 2010-2015



Greece

The vast majority of reported WNV cases in Greece across the six-year researched period were near regions bordering coastlines (Table 26). 100% of infections reported were approximating coastlines for both 2013 and 2014; 2010 (99%) and 2012 (98%) also observed a similar pattern. Only in 2011, 14% of the cases were in non-coastal regions, however, as much as 76% of cases were identified, similarly to other years, near coastal regions. Overall, 96.5% of reported cases were associated with coastal areas. Similar to Hungary, the vast majority of infections (over 81.8%) were traced in rural areas (Table 27, Table 24) followed by 16.5% in urban locations; this suggests that reported human WNV cases were three times more likely to occur in rural areas than those in urban locations. Only 2% of human WNV disease was found in intermediate areas making it the least likely landscape type to be associated with disease circulation in the country. This indicates that rural areas near coastlines were the most significant and prevalent human WNV landscape hotspots for Greece during

2010-2015. Its non-coastal regions and intermediate zones were the least effected across the study period, except for 2015 were no human WNV infections were recorded.

Table 26: Proximity of WNV affected areas to coastline (%), Greece, 2010-2015

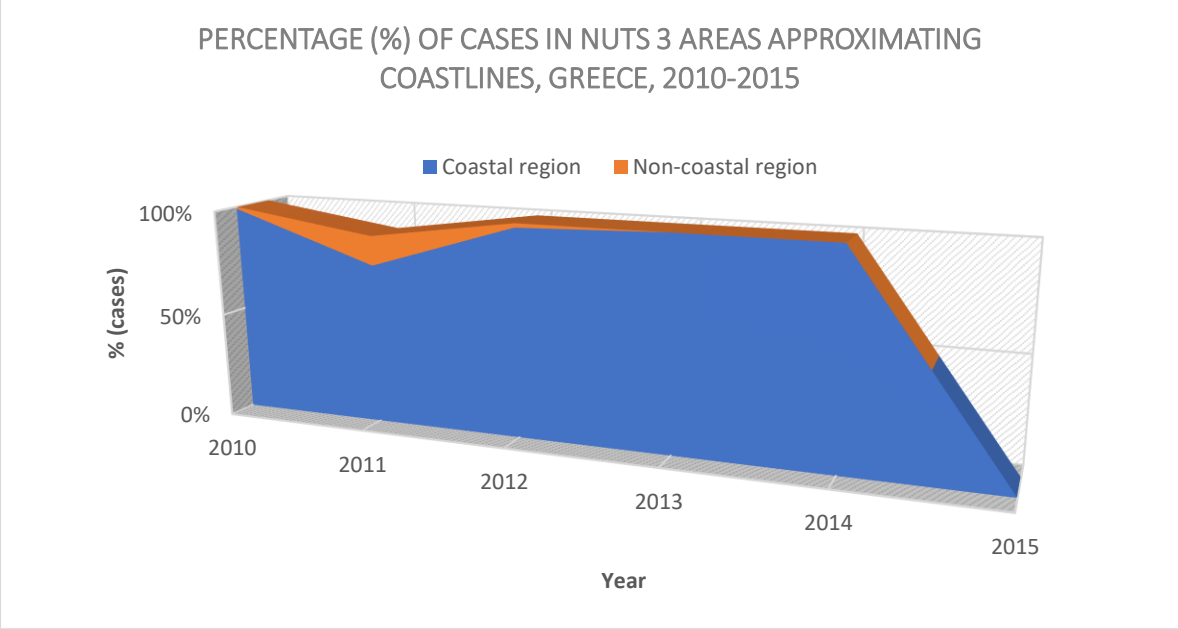
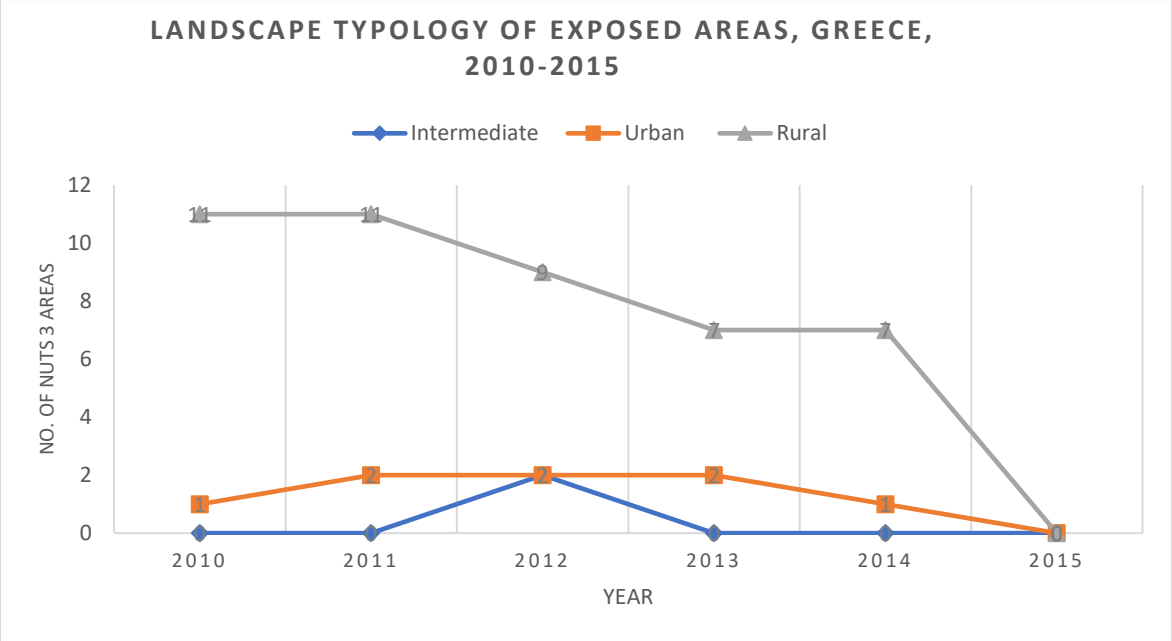


Table 27: Landscape typology (urban, intermediate or rural) per number of WNV exposed areas (NUTS3), Greece, 2010-2015



Italy

Italy had higher levels of human WNV infections in coastal areas during the first three years (2010-2012) and non-coastal regions in subsequent years (2013-2015) (Table 28). In 2014, 100% of cases occurred in non-coastal locations whereas in 2011 and 2012 respectively 93% of reported cases occurred near coastlines. The contrast was less extreme in 2013 of all years where less than two thirds (66%) of recorded cases were in non-coastal regions. These numbers show an almost even division between coastal and non-coastal regions, however 51.3% of cases occurred in non-coastal areas.

Unlike Hungary and Greece, the vast majority of human WNV cases were predominantly reported in intermediate landscape typologies (total of 62.5%) (Table 24); the most severe contrast was seen in 2013, were 11 of the 15 regions were human WNV infections detected were intermediate. Further, during the study period between 2010 and 2015, of human WNV cases 7.3% occurred in rural areas and 30.3% in urban locations.

These data suggest that across the 6-year period investigated in this study, the reported cases were almost two times more likely to be in intermediate territories compared to other locations especially urban locales. Although there were more rural areas reporting cases, the number of cases was higher in intermediate zones which may have affected the result (Table 29). In total, most infections took place in non-coastal (albeit only marginally) intermediate zones were the most likely epicentres for WNV transmission for Italy. Although most infections took place in intermediate zones, there were more rural areas reporting infections (Table 29).

Table 28: Proximity to coastline of WNV affected areas (%), Italy, 2010-2015

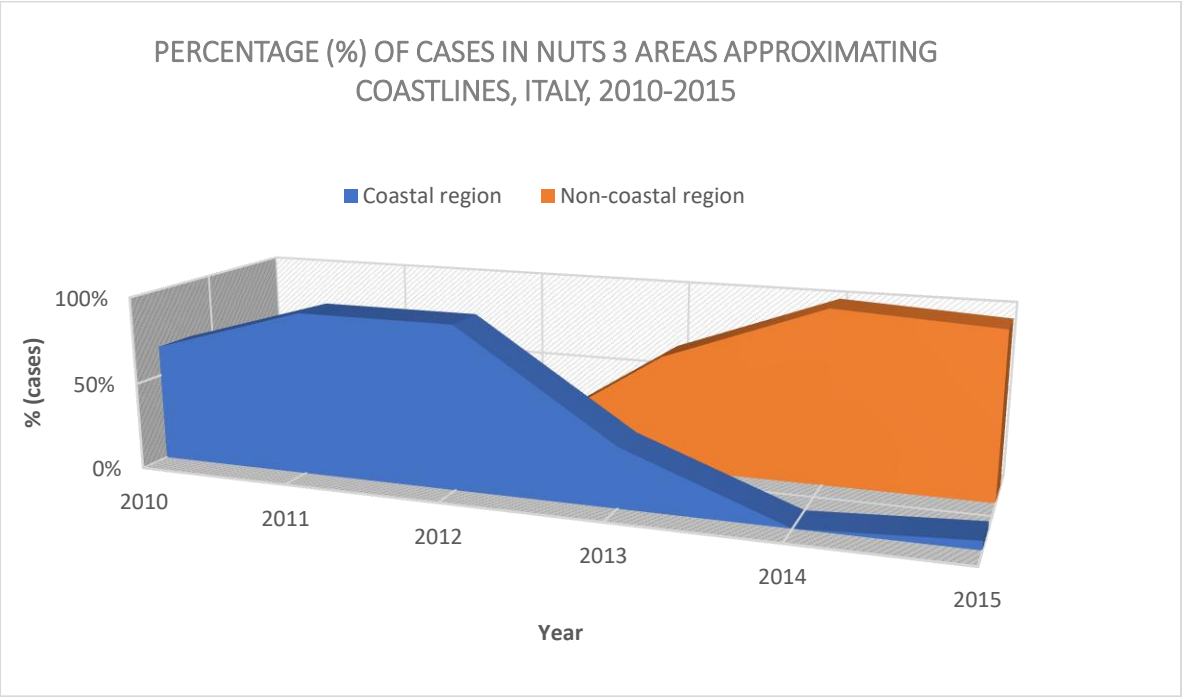
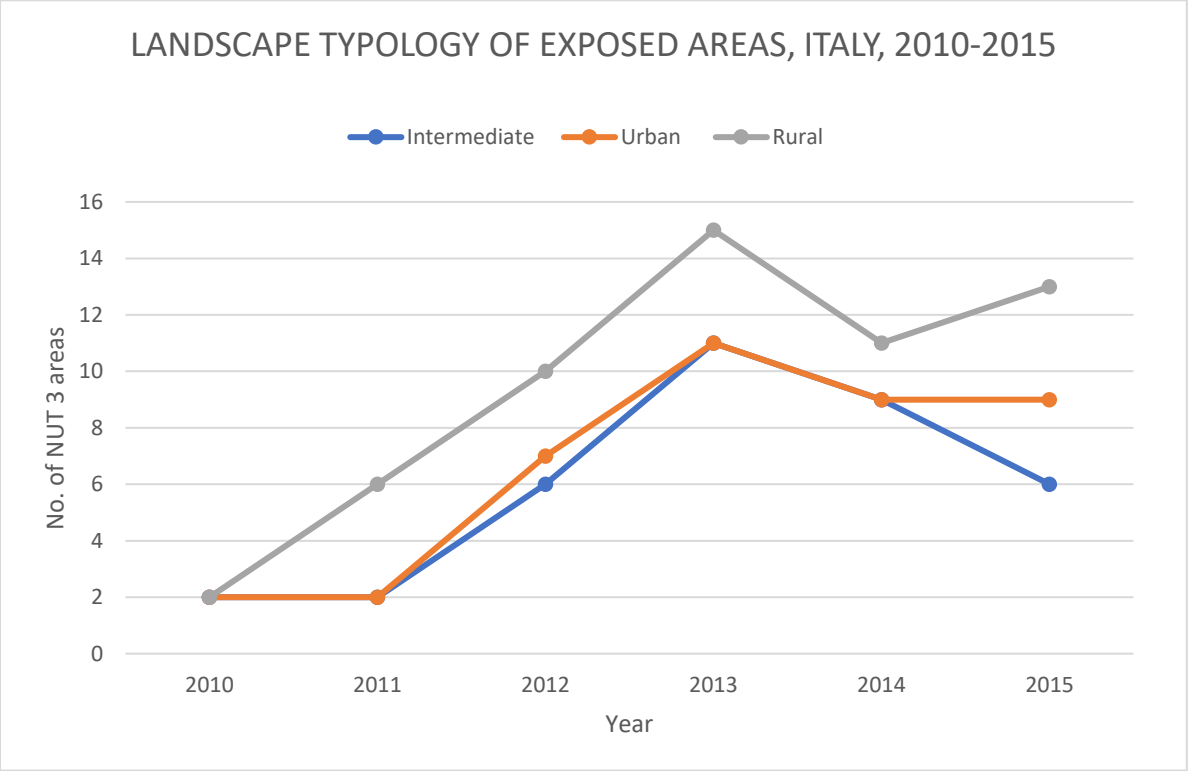


Table 29: Landscape typology (urban, intermediate or rural) per number of WNV exposed areas (NUT3), Italy, 2010-2015



Romania

Alike Hungary but in contrast to Greece's landscape associations with human WNV transmission, around 89.3% of WNV infections reported in Romania between 2010 and 2015 were detected in non-coastal regions (Table 30). In 2012, 100% of reported cases were in non-coastal areas. For 2013-2015, over 92% of detected infections were also found in regions not bordering coastlines. The same pattern was observed in earlier years, where in 2010 (81%) and 2011 (73%) were in non-coastal places, but the differences were less severe than in subsequent years.

Over 42% of infections in Romania during the 6-year period were detected in intermediate zones and 39% in rural areas (Table 24 & 31); this is similar, in terms of typological frequencies, to Italy but less severe. In 2011, 54.4% of cases were recorded in urban areas which was the highest score for that type of landscape typology across the 6 years. In 2013, 62.5% of cases were traced in intermediate areas at variance with the following year (2014) where only 37.5% of cases were linked to the latter and 58.3% to rural regions. These results suggest that most infections occurred in intermediate (albeit only slightly more than rural) areas not nearing coastlines as the most frequently detected topological hotspots for human WNV transmission in Romania for the period between 2010 and 2015.

Table 30: Proximity of WNV affected areas to coastline (%), Romania, 2010-2015

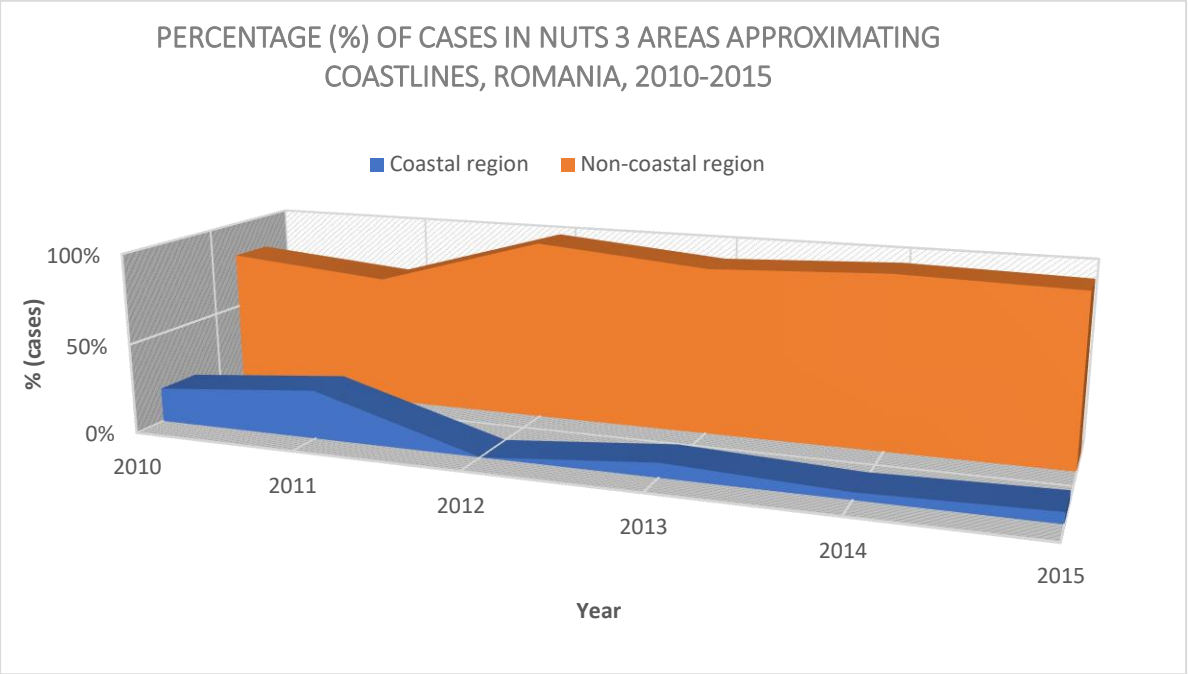
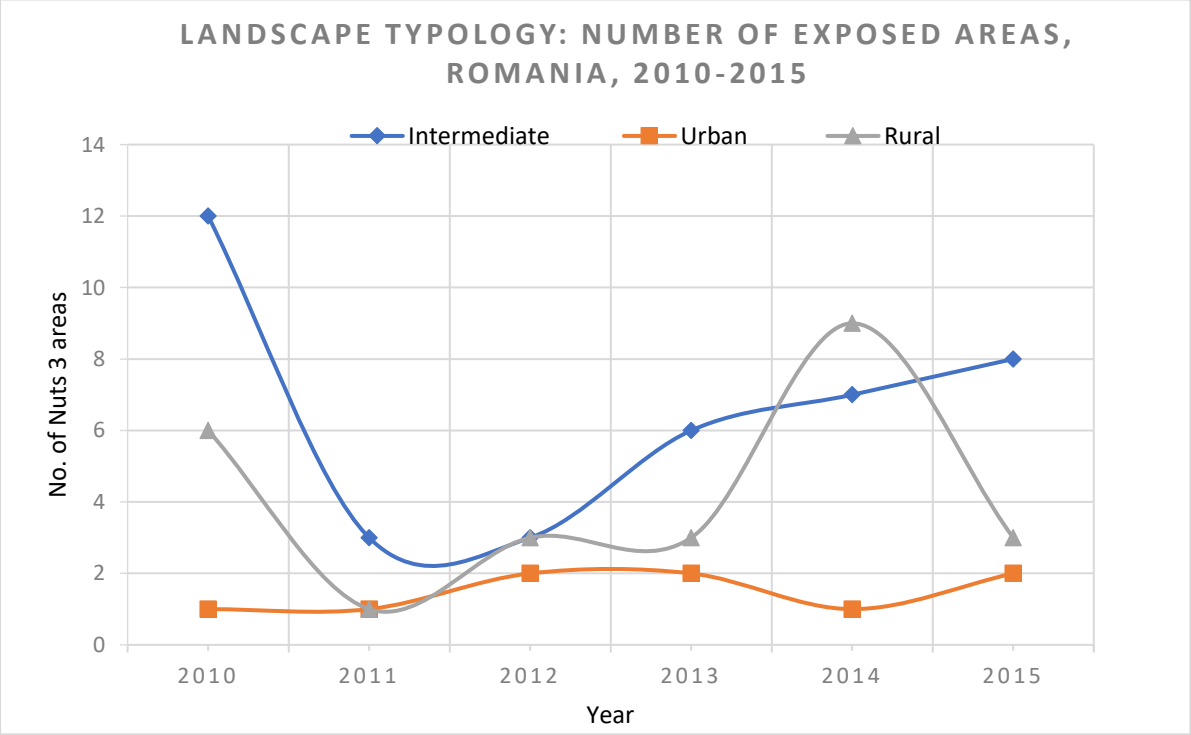


Table 31: Landscape typology (urban, intermediate or rural) per number of WNV exposed areas, Romania (NUTS3), 2010-2015



5.3 Inferential statistics and temporal lag-effects estimation by employing a Zero-inflated Negative Binomial Model (ZINB)

The third part of the analysis presents the findings of the probability of climatic factors influencing disease distribution over a three-month internal. The zero-inflation mixture framework employed in this study was made of two distinctive parts: a negative binomial and a point mass at zero. One examined all nine climatic coefficients and the dispersion parameter and the other the intercept and NUTS3 locations of reported infections as the regressor.

The ZINB model looked at the probability and associations between the occurrence of WNV human cases and the mean monthly climate and environmental values investigated in three different monthly lags (some month, one month before and two months before the WNV cases). The negative binomial regression (NBRM) formed part of the same statistical framework namely the zero-inflated mixture model. The count was a negative binomial regression (with log link). For modelling the unobserved state (zero vs. count) a binary process was used to capture the probability of zero inflation (binomial with logit link); this included an intercept and the NUTS3 location as the regressor. Parameters were estimated by maximum likelihood, and Pr(>|z|) set at <0.05 statistically assessed the significance of any associations.

A lag effect denotes the benefits of spacing with longer lags (Carpenter, Pashler and Cepeda, 2009). Some studies have shown longer lags to be more robust than shorter lags such as weekly (Rickard, Lau and Pashler, 2008). Thus, in this zero-inflated mixture model, the study looked at air temperature, dewpoint temperature, soil temperature, total precipitation, relative humidity, surface pressure, volumetric soil water, and wind components U (east facing wind velocity) and V (north facing wind

velocity) parameters' performance and association with WNV human disease distribution in three distinctive lags:

- Lag 0 are the environmental and climate conditions at the same month of an outbreak,
- Lag 1 are the environmental and climate conditions a month before the outbreaks and,
- Lag 2 are the environmental and climate conditions two months prior to an outbreak.

Lag 2, denoting environmental and climate conditions (averages) 2 months before a human WNV outbreak was recorded in the NUTS 3 unit, demonstrated the maximum log likelihood probability value (-1612) compared to the other two lags, lag 0 (-1823) and lag 1 (-1726). The Lag 2 model was therefore selected as the best model. Comparison by log likelihood is possible because all the three models have the same number of parameters, especially since the only difference between models is in the values of the covariates (the monthly average). The intercept and location (NUTS 3) were not statistically significant. Seven out of nine climatic coefficients, namely air temperature, dewpoint temperature, U wind velocity, total precipitation, relative humidity, surface pressure, volumetric soil water as well as the dispersion parameter (theta) were highly significant (under the set p-value of 0.05) (Table 32). The V wind component (north facing wind velocity) and soil temperature were the only climatic parameters which were not statistically significant in the best fitting model. The same was found for the intercept and location (NUTS 3) where no associations were reported. The volumetric soil water, dewpoint temperature and precipitation coefficients were the most significantly different from 0.

Table 32. Negative Binomial Regression model: Log-likelihood -1612 (two months before outbreaks (Lag 2)

Parameters (lag 2)	Estimate	Std. Error	z value	Pr(>|z|)
2 metre temperature, t2	-1.10	0.25	-4.37	**<0.001**
2 metre dewpoint temperature, td2	1.53	0.23	6.78	**<0.001**
Soil temperature level 1, so2	-0.19	0.12	-1.56	0.12
Relative humidity, r2	-0.51	0.06	-8.27	**<0.001**
Volumetric soil water layer 1, v2	6.52	1.89	3.45	**<0.001**
Surface pressure, sp2 (sqrt)	-0.10	0.02	-5.56	**<0.001**
10 metre U wind component, wu2	0.31	0.11	2.80	<0.005
10 metre V wind component, wv2	0.09	0.10	0.92	0.36
Total precipitation, tp2	755.31	156.41	4.83	**<0.001**
Theta	-2.53	0.22	-11.67	**<0.001**

Zero-inflation Binomial model: two months before outbreaks (Lag 2) with logit links

Parameters (<2 month lag2)	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	-2.37	2.96	-0.80	0.42
Nut3 ID	0.01	0.03	-0.48	0.63

A collinearity test (Annex B & Table 33) was also conducted to ensure that the interpretation of the model coefficients was not hampered by certain variables being exactly correlated. In the best fitting model, the regression coefficients were mostly uniquely determined as dewpoint temperature and air temperature had a moderate positive relationship and only soil temperature had a very strong positive relationship with some factors (air temperature and dewpoint temperature). However, soil temperature was included in the model given that:

- a high correlation between different temperature variables was expected given that all temperature variables use the same measurement scale,
- the parameter is not sufficiently researched (section 3.6.2.2), neither individually nor in a model combining other forms of temperature, in a human WNV disease context,
- “the fact that some or all predictor variables are correlated among themselves does not, in general, inhibit our ability to obtain a good fit nor does it tend to affect inferences about mean responses or predictions of new observations” (Kutner et al., 2005, p289, 4th Edition) and,

- the parameter was not statistically significant in the best fitting model and as a result had no influences on the interpretation of the model coefficients

- In zero inflated models, correlation between variables is not an issue if not accompanied by proportionality in the coefficients (something not recalled in our analyses) (Cincotta, Kevin and Lee. Multicollinearity: Coping With The Persistent Beast (2007 DoDCAS)).

Table 33: Collinearity Test- summary of variables in Best Fitting Model (Lag2)

Variable1	Variable 2	Correlation
Dewpoint temperature	Air temperature	0.6 (Moderate)
Soil temperature	Dewpoint temperature	0.9 (Very high)
Soil temperature	Air temperature	0.9 (Very high)

Although Lag2 was the best fitting model, the studied climatic parameters demonstrated a variety of associations across all three nested models. Table 34 shows the levels of statistical significance for each parameter across all 3 nested models.

Table 34: Associations of climatic parameters with WNV human infections, 2010-2015

Parameters	Lag 0	Lag 1	Lag 2* Best Model
2 metre temperature	No	Significant	Highly significant
2 metre dewpoint temperature	No	Highly significant	Highly significant
Soil temperature level 1	Highly significant	No	No
Relative humidity	No	Highly significant	Highly significant
Volumetric soil water layer 1	No	Significant	Highly significant
Surface pressure	Highly significant	Highly significant	Highly significant
10 metre U wind component	Highly significant	Significant	Highly significant
10 metre V wind component	No	No	No
Total precipitation	Highly significant	No	Highly significant
Theta	Highly significant	Highly significant	Highly significant
Intercept	No	No	No
NUTS 3	No	No	No

Note: * Best Fitting Model Note: No significant: p-value>0.05, Significant: p values between 0.05 and 0.01; Highly significant: p-value<0.01

Given that the other nested models (lag0 and lag1) also reported strengths of association with human WNV outbreaks, this section provides an overview of the main findings to potentially inform parameter-specific comparisons. Specifically, the ZINB model showed that during the same month of outbreak (lag 0) (Table 35) several climatic parameters, namely soil temperature, surface pressure, U wind component and total precipitation, were highly significant with a p-value of 0.05 or less. The dispersion parameter (theta) was equally significant; however, lag 0 had the lowest loglikelihood value (-1823) compared to the other two nested models: lag 1 and lag 2. The volumetric soil water and precipitation coefficients were the most significantly different from 0; the remainder eight factors were close to zero. Neither the intercept nor the NUTS3 predictor were statistically significant and both marginally below the average mean respectively.

Table 35. Negative Binomial Regression model: Log-likelihood -1823 (same month of outbreaks (Lag 0))

Parameters (0-month lag 0)	Estimate	Std. Error	z value	Pr(>|z|)
2 metre temperature, t2	-0.38	0.22	-1.75	0.08
2 metre dewpoint temperature, td2	0.08	0.18	0.42	0.68
Soil temperature level 1, so2	0.44	0.10	4.46	**<0.001**
Relative humidity, r2	-0.04	0.05	-0.82	0.41
Volumetric soil water layer 1, v2	0.88	1.66	0.53	0.60
Surface pressure, sp2 (sqrt)	-0.12	0.02	-7.76	**<0.001**
10 metre U wind component, wu2	-0.43	0.11	-4.02	**<0.001**
10 metre V wind component, wv2	0.00	0.09	-0.01	1.00
Total precipitation, tp2	-513.80	138.70	-3.71	**<0.001**
Theta	-2.81	0.30	-9.40	**<0.001**

Zero-inflation Binomial model: same month of outbreaks (Lag 0) with logit links

Parameters (0 month- 0 lag)	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	-5.04	57.52	-0.09	0.93
Nut3 ID	-0.01	0.15	-0.07	0.95

Lag 1 model examined the same nine climatic predictors, dispersion parameter and loglikelihood a month before the respective outbreaks were recorded. Air temperature, dewpoint temperature, relative humidity, surface pressure, volumetric soil water, and wind components U (east facing wind velocity) as well as the dispersion parameter

were all statistically significant. The model showed that soil temperature, total precipitation and wind components U (east facing wind velocity) could not predict an association reporting p-value > 0.05 (Table 36). This time series (lag 1) did not yield the maximum value but instead had the second-best log likelihood value (**-1726**) compared to the other two nested models: lag 0 and lag 2. As with Lag 0, the volumetric soil water and precipitation parameters were the most significantly different from 0, the remainder eight factors were close to zero. Neither the intercept nor the location (NUTS 3) proved to be significant predictors of disease distribution and both factors were below the average mean respectively.

Table 36. Negative Binomial Regression model: Log-likelihood -1726 (one month before outbreaks (Lag 1)

Parameters (<1 month 1 lag)	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
2 metre temperature, t2	-0.47	0.23	-2.07	0.04
2 metre dewpoint temperature, td2	0.66	0.20	3.27	0.01
Soil temperature level 1, so2	0.03	0.11	0.32	0.75
Relative humidity, r2	-0.22	0.05	-4.14	**<0.001**
Volumetric soil water layer 1, v2	3.57	1.73	2.07	0.04
Surface pressure, sp2 (sqrt)	-0.15	0.02	-9.11	**<0.001**
10 metre U wind component, wu2	-0.27	0.11	-2.49	0.012
10 metre V wind component, wv2	0.07	0.09	0.80	0.42
Total precipitation, tp2	-282.54	145.14	-1.95	0.05
Theta	-2.38	0.18	-13.13	**<0.001**

Zero-inflation Binomial model: one month before outbreaks (Lag 1) with logit links

Parameters (<1 month - lag1)	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	-1.69	1.29	-1.32	0.19
Nut3 ID	-0.02	0.03	-0.64	0.53

CHAPTER 6- DISCUSSION

This study found that WNV distribution in European human populations is not homogeneous in countries (namely Hungary, Greece, Italy and Romania) experiencing regular epidemic outbreaks with WNV human infections covering a diverse geographical and landscape range in Europe. The study explored those European countries experiencing consecutive epidemic outbreaks specifically where the disease spread over multiple areas and individuals reported ill at the same time over the years. The delineation between endemic, epidemic (outbreaks), hyperendemic and pandemic events is not clearly defined within the EU and how these apply to or differ in different mosquito borne and other infectious diseases. A unified or disease specific approach may further inform future assessment of the situation in countries where WNV and other virus circulate. Although outside the scope of this study, a critical question that requires future analysis is how mosquito borne diseases like WNV and zoonoses in general fit into a pandemic description (Gislason, 2013). A number of other EU countries, like Croatia and Austria, experienced endemic and/or sporadic cases which were not in scope for this study as the emphasis was placed on consecutive outbreaks. In addition to gathering data and monitoring events for example through the ECDC's Threat Tracking Tool (TTT), classification of the type/level of disease occurrence could further inform potential conclusions.

EpiMaps on European countries reporting recurrent cases can help better understand the spatiotemporal distribution of WNV; this can allow timely vector control measures to be activated and laboratory screening of blood and organ donations conducted, as a means of potentially reducing the risk of WNV human transmission. The incidence rate in this study captured both the coverage and rate of reported infections in each country; however, census data represent population estimates at a given time rather

than exact figures which presents a limitation. Further, the epidemiology of WNV infections in Europe is likely to be a result of diverse factors (e.g., age) (Lim et al., 2011) and variations in national, passive or active, surveillance systems (Kramer et al., 2008). In the US, Petersen et al. (2012) estimated that around 40% of WNV infections are reported; in Europe, active surveillance is compulsory in only 22 countries (ECDC, 2017) although WNV is a notifiable disease in Europe according to the EU case definition (European Commission, 2018). The full magnitude of the spatiotemporal distribution of the virus in European populations remains unknown with TESSy capturing only a small proportion of infections, those reported by hospitals and laboratories, with asymptomatic cases difficult to capture. Surveillance practices in the EU vary (Gossner et al., 2017) which may yield variations in reporting including underreporting; however, countries tend to adopt different systems to reflect national priorities and population needs. Interestingly, Greece, Hungary and Italy issue awareness alerts following the detection of equine cases as a means of protecting public health (Gossner et al., 2017; Rizzo et al., 2016; Szentpáli-Gavallér et al., 2014). The ECDC has recently started publishing weekly reports combining data on the distribution of human WNV infections across Europe and neighbouring countries (ECDC, 2017). Given that there is a vaccine in place for equids, albeit no central data is available on its uptake, herd immunity may increase in equids which makes their infections a weak long-term predictor for human disease.

Further, recent evidence from Greece and Italy shows that WNV disease presence in equids does not predict or is replicated in human populations in the same hotspots (Young et al., 2019); nevertheless, epizootic transmission, entomological and molecular surveillance of emerging strains with increased virulence may signal a potential public health risk and need for prompt blood safety actions. Italy is the only

country that has adopted an interdisciplinary approach to surveillance which is utilised to prevent human health risks (Rizzo et al., 2016) whereas Greece implements an active surveillance which includes case tracing whereas the remaining two countries perform passive surveillance (serological and virological examinations). These approaches may explain Italy's moderately low incidence rates observed over the studied period of 2010-2015, decreasing rates from Greece over time and variable incidence rates for Hungary and Romania. As notification rates and practices vary per country underreporting is difficult to systematically tackle unless surveillance and monitoring approaches are relatively similar. In the absence of active surveillance, predictive localised epidemiological models should be explored and updated on a bimonthly basis (as per the ZINB model's findings) as a means of preventing future outbreaks in regions where disease is in circulation. This would create an active barrier for continuous disease transmission especially if WNV is (becoming) permanently located in an area. Central funding and a commitment from the EU to create such models may allow their rapid development given the health and economic challenges some of the affected EU member states are facing as a result of WNV and other infectious diseases.

The absence of cases in Greece in 2015 could be a result of changes in surveillance practices which would explain the drop in the incidence rate from 8% to zero over the years. In countries or areas where there is recurring human disease, a subsequent absence of human WNV disease may be associated with the present of asymptomatic human cases therefore central guidance on strengthening the reporting practices of non-clinical cases may be required. This is vital as the viral load present in those cases is unclear. In fact, although reported human cases confirm viral presence in a given area or country, no reported/recorded cases of WNV infections does not necessarily

indicate disease absence. This is particularly relevant as most cases (>80%) are asymptomatic (ECDC, 2019; Rossi et al., 2010) and therefore not all cases will be captured by the relevant surveillance systems. Although vertical, interhuman (through blood or transplant donations) and travel acquired WNV infections have been reported across the period investigated, they count for a small proportion of the overall epidemiological state to influence the validity of the study results or be considered as a limitation. The exclusion of travel related infections from this study provides a more accurate representation of the epidemiological situation of the reporting country, unless the infection occurred in another European country which provides a more accurate depiction of the actual size of infection in the country were the infection was acquired. This distinction may require further exploration.

The infection rates observed during 2010–2015 are largely consistent with the disease burden reported elsewhere (ECDC, 2016). However, the occurrence of WNV epidemics in Hungary, Greece, Italy and Romania in continuous years during the researched six-year period suggests possibly new epidemiological developments of WNV infections in Europe; for example, the increased detection of WNV transmissions in South-Eastern Europe in consecutive years may be linked to:

- a permanent WNV endemisation in a region or country since its re-emergence,
- increasing awareness to virus,
- changing epidemiology,
- climatic and environmental conditions,
- genetic mutations and/or
- enhanced surveillance rather than signifying a novel outbreak of the virus. This will need further analysis to accept or reject as a hypothesis.

In Europe, WNV human infections associated with Lineage 1 generally produced either asymptomatic, mild febrile, severe or fatal disease which demonstrates an important association between the genetic makeup of the virus or disease phenotype (Schuffenecker et al., 2005; Lanciotti et al., 2002).

Following the re-emergence of the virus in 2008 with sporadic cases and epidemics across Europe, Italy, similarly to other countries, observed neuro-invasive disease in humans, three in 2008 and eighteen in 2009, all of which were part of the European Mediterranean/Kenyan cluster (Monaco et al., 2009). Phylogenetic studies revealed that the isolates were 98.8% similar to those strains circulating in its central region in previous years, particularly in 1998 (Monaco et al., 2009; Savini et al., 2008). Although the vast majority of endemic and epidemic episodes in Europe are linked to the European Mediterranean/Kenyan cluster, 73.7% human cases reported in 2008 in Hungary correlated with the Israeli/American cluster (Krisztalovics et al., 2008).

Lineage 2 strains have been first identified in Europe in Austria in 2008 and 2009 respectively and are similar to those found in sub-Saharan, North and South Africa (WOAH, 2008). Subsequently, Greece also confirmed circulation of lineage 2 strains following serological analysis of samples from two blood donors, pools of *Culex* mosquitoes and wild birds in 2010 and 2011 of similar homology to the Hungarian and South African strains detected in 1990s (Papa et al., 2010; Valiakos et al., 2010). Although previous serosurveys carried out between 2006 and 2010 indicated the presence of WNV antibodies in approximately 1% of humans (Papa et al., 2010), it was only in 2010 when the confirmation of its circulation was properly documented in humans in Greece.

A third lineage, which has been linked to Rabensburg virus, which is closely related to WNV, was found in the Czech Republic in 1997 and 1999 respectively (Hubálek et al., 2000); so far, no human disease has been linked to lineage 3 in Europe, however, experimental studies have demonstrated that it has the capacity to infect mosquitoes (Bakonyi et al., 2005; Aliota and Kramer, 2012). Two further independent lineages have been proposed which comprise isolates from Caucasus -Lineage 4- and India -Lineage 5, previously considered clade 1c of lineage 1- respectively (Chowdhury et al., 2014; May et al., 2011; Platonov et al., 2011; Bondre et al., 2007; Lvov et al., 2004) for Europe. There is no current[26] evidence that lineages 4 and 5 are circulating in Europe.

These developments further stress the ability of the WNV to adapt, circulate and affect humans and other hosts (Calistri et al., 2010). They present an additional challenge to public health management but data on genetic associations is not systematically produced or monitored. This creates an additional gap considering that it has also been proposed that three further genetic lineages may now co-exist. It is unclear from reviewing the literature which of the two genetic lineages of WNV is the most prevalent and severe in Europe which requires further research and data availability.

West Nile Virus can lead to topical endemic outbreaks or extensive epidemics in human populations with the genetic composition of the circulating strain playing a key role in these epidemiological disease expressions (Monini et al., 2010). However, due to a number of limitations such as capacity, availability, timeliness or scientific advancements in knowledge of new lineage and molecular analyses, information on the genetic make-up of circulating strains is often scarce. The latter may allow the

[26] at the time this study was conducted (2019)

composition of new European risk models capable of capturing an expansion of traditional infection boundaries, larger population bandwidths and more diverse habitats. Additionally, national guidelines for localised surveillance, learning and reporting systemic enhancements can improve the assessment of disease burden and better forecast disease spread and severity. Further efforts are therefore required to root cause and unearth the origins of the emerging epidemiological morphology of successive WNV infections in Europe to allow timely and relevant responses to the threat posed by WNV on human populations.

Moreover, due to the surge and global distribution of other flaviviruses, such as Japanese Encephalitis, Zika and Dengue viruses (Lustig et al., 2018; Mackenzie et al.; 2004), more sensitive tests would be required for the detection of WNV which would allow to differentiate between different mosquito borne diseases and ultimately lead to the development of a WNV vaccine for human use. In fact, a commercial vaccine for human use entered phase II of a clinical trial but never materialised due to uncertainty around the potential target population to receive the vaccine (Kaiser, 2012). Other developments such as reports of high prevalence of chronic kidney disease among younger populations infected by WNV (Nolan et al., 2012) and presence of the latter in urine years after initial infection (Murray et al., 2010) call for a renewed emphasis and urgency into prioritising a human vaccine against WNV. An age-targeted vaccination program has been recently recommended as a viable cost-effective response to WNV (epidemic) outbreaks (Shankar et al., 2017). Gender, age, environmental and seasonality explored in this paper make a strong case for a revived commitment to identify suitable therapeutics and vaccines to address an increasing public health threat. Specifically, it supports the efforts of better understanding who may fall under the at-risk groups and provides specific information on age, gender and

other correlating parameters in countries experiencing epidemic outbreaks to inform policies aimed at identifying age and other target groups.

The emergence of other mosquito borne flaviviruses, such as Chikungunya (CHIKV) and Rabensburg, may create additional pressures for European authorities managing the spread and transmission of infectious diseases (Beltrame et al., 2007; Bakonyi et al., 2006). CHIKV, which originates in south-east Asia, was previously only found in imported cases in European countries, however, in 2007 Italy reported 131 cases of Chikungunya arboviral fever and there is now evidence that this virus, alike WND, may soon become permanently located in southern Europe (Mondini et al., 2007; Pialoux et al., 2007; Beltrame et al., 2007). Further, blood (donation) screening for WNV unintentionally identified an expanding Usutu virus circulation in Italy, in the Lazio region, between 2017 to 2018 (Carletti et al., 2019).

With the emergence of the Rabensburg virus in the Czech Republic, which is also closely related to WNV, and parasitic infections, such as Malaria, transmitted through mosquito bites also being reported in countries of the Europe Union (WHO, 2013), the risks to public health from infectious diseases are becoming even greater. Further, a synchronous circulation of a diverse set of arboviral infections may lead to further and more complex mutations which highlights the need for rigorous surveillance in areas where these viruses are active but calls for an improved understanding of the differences and/or similarities in geo-temporal patterns and key drivers; this is particularly important from a West Nile Disease management perspective as treatments currently available are only supportive. These developments underline both the complexity and importance of WNV monitoring and surveillance.

This study also looked at age as a whole and identified the WNV disease incidence was higher with increasing age which tallies with other studies' findings (Yeung et al., 2017; Lindsey et al., 2010; Pauli, 2004). The highest ASR in EU/EEA was in 2010 (0.08 cases per 100,000 inhabitants) and lowest in 2014 (0.02 cases) despite Greece reporting no cases in 2015.

The study, however, has some limitations in terms of disease associations to age. Firstly, a link between WNV and other conditions such as heart disease, liver disease, dementia, immunodeficiency and autoimmune disease has been found in previous studies (Lindsey et al., 2003; Green et al., 2000). However, TESSy data do not include information on concurrent conditions and/or health status of infected individuals in the study population which may be associated with certain age groups being more prevalent. Analysis of such data can enable a better assessment of whether WNV infections and increasing age correlate.

Secondly, resistance to infection is subject to a variety of factors with age one the risk factors associated with neurological disease and greater WNV replication by triggering certain immune responses (CDC, 2016; Williamson et al., 2016; Hasbun et al., 2016; Montgomery and Murray, 2015; Gray and Webb. 2014; Hayes et al., 2005). Human immunosenescence contributes to morbidity and mortality in later life thus the immune response to WNV infection in elderly groups can provide useful information on their innate and adaptive immune responses to West Nile disease (Montgomery and Shaw, 2015).

TESSy data provided limited information on disease severity and clinical outcomes which would have enabled a better understanding of WNV manifestation in each age group especially those 65 and over which appeared to be most affected; this can

inform potential paths for therapeutic approaches and contribute to the knowledge base of immune susceptibility to WNV and other flaviviruses in elderly populations. This would also be of relevance to a plethora of neuro-tropical diseases given that the world's population is ageing (UN, 2013).

Regarding West Nile Virus (locally acquired cases) gender distribution in affected EU Member States, 2010-2015, Legato (2004) suggests that the risk of infection in men is slightly greater compared to women across countries; however, this study identified that across the years and countries, locally acquired West Nile Virus cases were statistically significant (p-value <0.5) and considerably higher in men than women (55.6% in 2010, 62% in 2011, 57.6% in 2012, 60.1% in 2013, 69.2% in 2014 and 73.4% in 2015). Most academic studies reporting data on the rate of hospitalisation (Curren et al. 2018; Gubler, 2007; Mostashari et al. 2001) per gender report male infections. However, continuous reporting of European data (ECDC, 2015) and from the US (CDC, 2015) suggests that this is a universal observation in terms of incidence rates with the sex ratio (male: female) being 2.1. Although, hormonal differences (Kovats 2015), OAS1, dysregulation of TLR pathways and IFN-α production (Ziegler et al. 2017; Kong et al., 2008) have been explored as potential triggers for this variation in viral infection rates between males and females, the precise cause remains unknown. Also, Legato (2004) suggests that men are more likely to work outdoors which may increase infection rates in male populations. Overall, the age structure of a population affects a nation's key socioeconomic behaviours which may contribute to gender differences; this requires further investigation in terms of the epidemiology and public health impact on disease management.

This research showed that 2014 and 2015 recorded the highest rates of WNV infections for men compared to women across the EU/EEA; in fact, there may be a link between Greece's low reporting numbers in 2014 and absence of cases in 2015 and Italy's consistently high percentage of male infections. Therefore, more analysis is required to produce more stratified evidence. Additionally, a longitudinal as well as prospective investigations could provide greater and more confirmatory statistical power as regards the correlations between disease rates and gender attributions.

Over 60% of cases reported by Hungary from 2010 to 2015 were male between 45-64 years of age. In addition to genetic variations, its population structure and age distribution for men is the largest for those 25-54 years (42.01%) (Index Mundi, 2019) which may have influenced the results. Hungary's lowest median age for both females (49) and males (56) observed across all studied countries may also support this hypothesis, but further analysis is needed to evidence the latter.

69% of the 323 cases reported during the 6-year period by Italy over the 6 years were male over 65 years; however, females account for most of the population share for age groups 25 and over, especially for those 55 and over. Those 65 years and over make the country's second largest age group (21.69%: male 5,817,819/female 7,683,330) however, only the age group of 0-24 years is dominated by males in terms of population share (Index Mundi, 2019). In this case, it can be argued that the share of population between males and females does not directly influence WNV infections, therefore other underlying factors may exist.

As regards Greece, 59% of the 623 cases between 2010 and 2015 were male over 65 years. Greece has a similar population structure per gender to Italy with men occupying a larger share of the population in 0-24 years age groups; although there

are more women aged 65 years and over (male 997,359 /female 1,277,871), this age group accounts for the second largest (21.14%) compared to all other groups which may explain the highest median age for both women and men (Index Mundi, 2019).

Around 60% of Romania's 162 cases across the 6-year studied period were male over 65 years. Women account for a higher share of the country population for only two distinct age groups 55 and over (male 2,698,989/female 3,551,794) (Index Mundi, 2019) which may explain the highest median age for females and lowest for men for those groups. Although those (both males and females) aged 65 years and over are the second largest population group which may justify to some degree the age and gender findings, more research is needed to understand men's behavioural, genetic and socioeconomic differences to explain the higher comparative rate of WNV infection.

The study also looked at a variety of environmental, namely territorial and landscape, features to assess their relationship with WNV disease occurrence in human populations. Although country level environmental links with viral presence were identified, the picture across all countries was not homogenous. Further, direct comparisons with previous studies were not always available to confirm or reject the findings. The environmental parameters utilised in this study, specifically the proximity to coastal, or cases within urban, rural and intermediate zones, have not been collectively explored previously in countries experiencing consecutive human WNV epidemics.

There has been some evidence (Vu et al., 2017; Shukia et al., 2012) that suggests that those living in coastal areas are more likely to contract West Nile Virus. This study showed human West Nile disease transmission between 2010 and 2015 occurred in

both coastal and non-coastal areas. Specifically, 63.7% of cases across the four studied countries were detected in non-coastal areas; this is likely to have been affected by the fact that Hungary has no coastlines and Greece reported no infections in 2015.

Greece's considerably higher infection rate (96.5%) in coastal regions across the 5 years may have influenced this heterogeneous overall result. Its geography and population distribution close to coasts may have played a role in this rate. For example, 33% of the inhabitants reside within 1-2 km of seashores and 85% no more than 50 km away of a coastal strip. It is noteworthy that most of Greece's economy revolves around its shorelines, with most of its large urban centres, including 80% of industry and 90% of tourism sector, approximating coast lines (Ministry for the Environment, 2006). Projected freshwater shortages and prolonged periods of drought may have shifted WN viral distribution to populations living even closer to its coastal zones, especially those regions with low precipitation levels such as the Greek islands and the region of Thessaly which experienced recurring WNV epidemics during the studied period. EU-funded projects, such as Sesame, studying the eco-system changes of the Mediterranean and Black Seas would need to consider the interactions between future disease and ecosystem evolution.

Overall, 51.3% of Italy's human WNV infections occurred in non-coastal areas which demonstrates that proximity to coastline is not a clear indicator for the country. Italy had higher levels of human WNV infections in coastal areas during the first three years (2010-2012) and lower in subsequent years (2013-2015). Of its 21 regions, 15 are coastal regions and Italian seashore is home to many residential and industrial centres. Alike Greece reduced rainfall is leading to desertification (European,

Commission, 2017) not only for the islands of Sicily and Sardinia but also Italy's southern and northern regions where consecutive epidemics have been recorded during the studied period. Additionally, an anticipated increase in saltwater dispersion into coastal freshwater basins (European, Commission, 2017) is expected to have a negative impact on agriculture; this form of degradation may have influenced population behaviours with WN disease also moving further away from those regions to predominantly urban and semi-urban and non-coastal. A better understanding of Italy's eco-system changes and relation to WNV circulation in human populations is required.

Of the human WNV infections reported in Romania between 2010 and 2015, 89.3% were detected in non-coastal areas with differences less severe in the early years (2010 and 2011) compared to the remaining four years. In 2012, 100% of reported cases were in non-coastal areas. For 2013-2015, over 92% of detected infections were also found in regions not bordering coastlines. The same pattern was repeated in earlier years, where in 2010 (81%) and 2011 (73%) were in non-coastal places, but the differences were less severe than in subsequent years. Romania's coastline stretches over 247 km, representing around 2,97% of its total territory, with 18 coastal areas 13 of which attract tourism (Simon et al., 2011); it has diverse biodiversity in coastal regions including the Danube Delta, the most significant wetland in Europe. Its significantly small coastal proportion perhaps is related to a comparatively low distribution of human WNV distribution in areas approximating seashores. A European Commission fiche on Romanian's climate change implications (European Commission, 2017) reported that in addition to flash floods, drought and desertification, beach erosions may be important risks affecting the country including its main coastline. This requires further investigation. It is unclear if climatic changes

near coastal zones play a role in the circulation of WNV near coastal areas. Previous research (Chai et al., 1998), detected that leaking pipes and standing water near urban settings allowed the formation of infestations which caused significant WNV outbreaks; this evidence corresponds with this study's findings that high percentage of WNV infections mainly occur in non-coastal and urban habitats.

Overall, the above findings suggest that proximity to or distance from coastlines is not a definite trigger of human WNV infections as results vary per country. However, for countries such as Greece and Romania there is a strong link between WNV circulation and coastal or non-coastal areas respectively. In addition to Hungary's lack of coastal zones, Italy's coastal typological associations were less robust and conclusive.

A number of studies have explored the relationship between certain landscape typologies and WNV transmission in birds, humans and mammals, the vast majority of which investigated the rural-urban interfaces in animal health (CDC, 2015; Paz, et al., 2013; Paz and Semenza, 2013, Hamer et al., 2008; Ruiz et al., 2007; Turell et al., 2005; Kilpatrick et al., 2005; Dauphin et al., 2004; Dohm et al., 2002; Campbell et al., 2002; McLean et al., 2001). An emphasis on human infections and landscape influences is scarce and predominantly explored at regional and to a significantly lesser degree at national/pan-European level (Chaskopoulou et al., 2016) with a clear gap in evidence on countries facing consecutive human WNV endemic or epidemic outbreaks; in order to address this gap, this study explored the environmental typologies, namely urban, rural, and intermediate, associated with human West Nile virus circulation in four epidemic-ridden EU countries over a 6-year period.

It identified a spatially heterogenous landscape-human infections association across the studied countries depicting a deviation in typological trends which historically

observed significant disease presence mainly occurring in rural areas near wetlands when WNV resurfaced in Europe in 1998 (Hubalek and Halouzka, 1998). Specifically, rural regions were more commonly associated with higher disease transmission across the years for Greece and Hungary compared to other areas whereas intermediate (peri-urban) zones observed the highest rates in Italy and Romania. However, all four countries reported infections across all three typologies. This suggests that the traditional rural boundaries of human WNV transmission have now been surpassed and disease distribution and landscape is not as homogenous as it was once hypothesised. This could be the result of a plethora of factors triggering intensive West Nile viral circulation and requires further investigation and validation as this can be a scale effect.

In addition to the heterogenous landscape-human infection relationship across countries, country population concentration and/or density in the three landscape typologies (Eurostat, 2018) may also have a close association with disease spread in human populations. For example, Italy's highest share of population by type of region resides in intermediate areas (43.7%) where most of the infections were recorded (62.5%) in as many as 11 of the 15 regions in 2013. Barzon et al. (2013) reported equally variable typological distribution of human WNV disease. Previous research has shown that in contrast to other species, the vast majority of female *Culex sp.* are found at canopy level rather than on land in natural habitats (L'Ambert et al., 2012; Becker et al., 2010) which may explain the increased rates observed in intermediate and urban settings.

Equally, most infections in Hungary were reported in rural areas which account for the country's highest population share (47.9%); similarly, Greece's evenly split share of

population between rural (44.2%) and urban (45%) may also explain the presence of WNV human disease predominantly in those settings (81.8% and 14.5% respectively) with only 2% of human WNV disease recorded in intermediate areas (which represents the smallest share of the total population (10.3%)) making it the least likely landscape type to be associated with disease circulation in Greece. Ladbury et al. (2013) also observed similar, albeit regional, distributions in 2013 in Greece.

Evidence from previous epidemics in Romania suggested (Platonov et al., 2001; Chai et al., 1998) a higher prevalence in urban areas and lower infection rate in rural locations. Romania's distribution of population between intermediate (43.9%) and rural (46.2%) populations matched the rates of WNV disease circulation (42% in intermediate zones compared to 39% in rural geographies) identified in this study. This suggests a typological shift especially in terms of rural areas. Given that the split between urban and intermediate locations was not previously available, it is not possible to say if previous areas grouped these typologies together.

To qualify these findings further comparative research is needed on historic and prospective data; there is currently no research that this study has identified that investigated European human WNV epidemics for all three typologies to make meaningful comparisons. Although a link between population density and viral presence may explain human WNV outbreaks in certain typologies in those countries, other factors such as the maintenance and condition of infrastructure may also be associated with certain mosquito breeding sites and as result have a role in disease distribution. A variety of other underlining factors may exist which would require careful consideration such as the quality of national, regional and/or local infrastructure conditions in those countries. For rural areas, the structure and/or condition of

channels, old river arms, big puddles, bogs, swamps and marches may have led to prevalent *Culex* mosquito breeding sites therefore increased human WNV infection rates. Farming, transport and proximity to such sites, could also explain the relationship between prevalent *Culex* mosquito breeding sites and high WNV human disease circulation. A recent study (Chaskopoulou et al., 2016) has shown that puddles, drainage channels, sewage and septic systems are linked to WNV epizootic circulation in intermediate zones and in underground parts in urban areas.

Human behaviour in terms of the application (or absence) of preventive and/or protective equipment and national guidance (or lack of) in those areas where infectious mosquitoes are circulating may also be contributing to increased viral replication in certain typologies. Further, previous research has shown that 50% of *Cx.pipiens* mosquitoes can transmit WNV to future hosts within 28 days (Eastwood et al., 2011). As a result, viraemic mosquitoes may be present in certain locations for a significant period of time capable of infecting multiple hosts thus country results in certain environments may be skewed by the presence of viral vectors in one location. For example, in 2014 disease was present in Hungary in only three regions which contributed to an increased overall rural country rate across the studied years. In Romania, the dominant typology varied each year, spanning across all three landscape classifications (urban, intermediate and rural) which could be linked to a variety of factors including mosquito abundance and certain location concentrations in that year. Recent entomological surveillance (Calzolari, et al., 2020) supports this theory detecting “intensive viral circulation in 2018, also involving urban areas which are usually less suitable than rural areas for WNV circulation”. Therefore, infected mosquito circulation causing enzootic, epizootic and/or zoonotic disease in certain typologies may also be capable of influencing the overall disease distribution in certain

zones. This would explain variations in typological exceptions such as in Italy in 2011, where there were more cases reported in rural rather than intermediate territories which is its dominant share of population. However, given the wide-ranging transmission timeframe associated with *Culex pipiens'* infectability, cases reported in a specific region may not truly correspond with the actual place an infection which could be ultimately distorting the final typological attributions.

Thus, the precise role of landscape typologies requires both human and vector WNV disease analysis to further understand whether increased human disease rates are also amplified by behavioural factors, atmospheric conditions and/or vector activity in those areas. Therefore, the landscape typology and population density analogy can be seen as a significant effector in disease presence in human populations but not as the sole or singular influencing factor of human WNV disease distribution; this is because other, either behavioural, vector sustainability, climate or infrastructural-conditions, may prevail which would require equal and preferably simultaneous investigation to produce robust associations. Overall, European human WNV epidemics during 2010-2015 showed landscape diversity compared to US data which showed that disease distribution concentrated around urbanized environments (Semenza et al., 2016).

A recent IPCC report (IPCC, 2013) introduced clearer than ever evidence that a variety of features of the climate system are shifting. The causes of WNV's widespread distribution are complex, but it has been frequently theorised that changes in climatic patterns and ecosystem characteristics affect WNV transmission. WNV is in fact the most extensively dispersed and highly adaptable arbovirus compared to many others also causing neurological disease in human populations (Chancey et al., 2015).

Several studies have previously utilised mathematical modelling techniques to identify how climatic related conditions facilitate or lead to the formation of human WN disease outbreaks.

Hartley et al. (2012) and Chevalier et al. (2014) assessed the climatic triggers of WNV and looked to identify modelling methodologies respectively. Most published work so far focussed on air temperature with precipitation and relative humidity the second most dominant factors explored, albeit some demonstrated very low associations with human WNV transmission (Paz et al, 2013; Hartley et al, 2013; Hartley et al., 2012; Soverow et al., 2009; Paz and Albersheim, 2008; Liu, Weng and Gaines, 2008; El Adlouni et al., 2007; Paz, 2006; Dohm, O'Guinn and Turell, 2002). The findings of these studies are inconsistent suggesting that the respective influence of each of these parameters is difficult to assess given the intense intricacy of zoonotic transmission cycles. Yet, modelling practices using consistent, diverse, robust and spatiotemporal specific atmospheric datasets can uncover how a wide range of meteorological conditions interact and correlate with human WN disease prevalence. This can create an epidemiological framework that looks at the influence of the climate holistically rather than focusing on monomeric influencing factors.

One of the aims of this study was to investigate how climate and human WNV disease interact spatial and temporal during and before an outbreak occurred. It utilised robust climatological modelling framework, namely the ERA-Interim re-analysis methodology, which provided homogeneous, detailed atmospheric spatiotemporal data for the NUTS3 areas experiencing WNV outbreaks. This is first time multiple and diverse meteorological variables were tested in three distinctive lag timeframes approximating the distinct outbreaks to unearth potential associations between the epidemiology of WNV human infections and climate. Historic data, between 2010-2015, based on real

observations of nine distinctive atmospheric parameters reanalysed from atmospheric modelling produced a novelty in our understanding of the underlying causes of outbreaks when combined with retrospective epidemiological vector-borne data on human transmission.

Specifically, the present study demonstrated that the consecutive outbreaks of WNV spread in human populations witnessed in Europe between 2010-2015 were linked to certain atmospheric and environmental parameters, in particular air temperature, relative humidity, dewpoint temperature, surface pressure, total precipitation, wind velocity, and volumetric soil water moisture, more so two months prior to an outbreak occurring. It supports previous findings suggesting an association between the diverse geographic expansion of WNV and certain climatic conditions (Paz and Semenza, 2013) but goes a step further to provide an appropriate lag timeframe for countries facing successive outbreaks. As such, one of the prominent outcomes and distinctive benefits of this study is the identification of associations between meteorological features and WNV human cases during an outbreak, two months before the outbreak has occurred, whereas most of other studies concentrated their efforts on mosquito density when researching the influence of climate in the context of WNV. These results may provide climatological spatiotemporal evidence to explain some of the triggers of the endemisation processes of WNV in humans in various Southern and South-eastern European locations.

The spread of the WNV human cases during 2010–2015 in European countries facing successive epidemics reveal reduced variability in duration and the time of occurrence but higher variability in number of cases across the 6-year period. Climatic conditions such as high air temperature in the summer 2010 may have led to mosquito

abundance and therefore explain an increased human disease prevalence (Stilianakis et al., 2016). The remaining years were climatologically more diverse but with high temperatures with 2014-15 particularly variable in terms of rainfall and temperature which may explain the marginal decrease in cases for Greece.

Table 37 summarises the positive and negative associations in the best fitting model. The following section presents an analysis of how the selected hydroclimatic factors employed in this study agree or not with findings from other WNV studies.

Table 37: Climatic parameters' positive and negative associations in the best fitting model, 2010-2015

Climatic Parameters	Lag 2- Best Fitting Model
2 metre temperature	Positive
2 metre dewpoint temperature	Positive
Soil temperature level 1	Negative
Relative humidity	Positive
Volumetric soil water layer 1	Positive
Surface pressure	Positive
10 metre U wind component	Positive
10 metre V wind component	Negative
Total precipitation	Positive

Ambient temperature was a very strong factor in the best model, slightly less so a month before an outbreak was recorded and not statistically significant for WNV infections in the month of the outbreak. There was no other study found to directly compare this outcome in monthly intervals although a clear link between temperature and outbreak severity in human populations has been reported by many other studies (Paz et al, 2013; Hartley et al, 2013; Hartley et al., 2012; Soverow et al., 2009; Paz and Albersheim, 2008; Liu, Weng and Gaines, 2008; El Adlouni et al., 2007; Paz, 2006; Dohm, O'Guinn and Turell, 2002). Air temperature contributes to the formation

of biological viruses and circulation of WNV by influencing the seasonal abundance and increase of mosquitoes hosts as well as variability in geospatial distribution of disease prevalence in human populations (Paz et al., 2013; Andrade et al., 2011; Kilpatrick et al., 2008; Kinney et al., 2006; Reisen, Fang and Martinez, 2006).

Elevated temperatures trigger an amplification of vector populations, shorten the intermission between blood meals and incubation length, quick viral replication and transmission to host populations (Paz and Semenza, 2013; Ruiz et al., 2010; Paz and Albersheim, 2008; Kilpatrick et al., 2008). Although Cornel, Jupp and Blackburn (1993) and Kinney et al. (2008) demonstrated that WV viral replication can occur in various temperatures ranging from 14°C in certain mosquito populations to 45°C in symptomatic birds, elevated temperatures expedited replication capacity (Jia et al., 2007; Kunkel et al., 2006). However, other studies have shown that high temperatures over 30°C can slow WNV mosquito amplification (Calistri et al., 2010), shorten the larval phase in certain *Culex* species (Reisen, 1995) and shift migratory avian patterns to early springtime therefore increasing host susceptibility (Marra et al., 2005; Cotton, 2003; Parmesan and Yohe, 2003). Additionally, previous evidence has shown that the period between exposure and disease symptomology in human populations can vary from 2–6 days to as much as 14 days (Petersen et al., 2003; Campbell et al., 2002). Thus, given the extended duration of incubation period and climatic influenced mosquito and larval activities, the findings of this study may lend renewed support to these theories which suggest that temperature in earlier months, when the latter is likely to be not as high on average, is a predictor of WNV disease circulation.

DeGroote et al. (2008) have previously observed a strong connection between dew point temperature WNV human disease prevalence but Soverow et al. (2009) reported

that an increase in WNV human disease prevalence was only associated with dew point over the subsequent 3 weeks rather than during the same week of an infection. Similarly, Steven et al. (2018) found a strong association from 4 weeks prior to sampling.

This study provided new evidence that dew point temperature was significantly associated with higher WNV incidence in human cases in the months preceding but not during the outbreak. Although the above study designs differ it terms of their designate timeframe, there is an indicative trend that indicates that dew point temperature is not liked to higher levels of infection during the reporting/recorded time. Nevertheless, and unlike air temperature, dewpoint temperature is not sufficiently researched as a parameter influencing WN disease circulation to establish a position of its impact on human health. The above results suggest that dew point temperature's performance and influence in the preceding and following weeks and/or months of an outbreak is important however they require further investigation to fully understand its role and significance in human WNV epidemiology. Given that months, as utilised in this study, cover a wider chronological timeframe, a repeat study looking over the next half of this decade (2016-2020), can further inform these findings. Nevertheless, these results provide evidence to inform future mosquito control efforts in those countries.

Soil temperature did not have a strong predictive capability in the best fitting model including one month before the WNV outbreaks were recorded. It was, however, a significant predictor during the month of the reported outbreak. These findings do not correlate with previous findings by Stilianakis et al. (2016) which demonstrated that soil temperature, followed by air temperature and soil water content, were the predominant factors influencing disease WNV distribution. In contrast, this study suggests that air and dewpoint temperatures are more related to the ecology of the

disease. It is unclear whether soil temperature has a different regional output dynamic compared to a country wide, however the findings of the present study were based on NUTS3 to indicate an altering discrepancy between the two studies. Therefore, changes in soil temperature may not be an important meteorological factor in influencing larval development or mosquito abundance in the months before a human outbreak. However, there is a strong correlation between air and soil temperature, and therefore combining both factors in the model may result in one of the two factors becoming insignificant. Additionally, findings may have been influenced by other factors, for example cases being based on dates of notification rather than actual disease occurrence. Future efforts may need to concentrate on developing a soil multi-model that looks as both hydrological i.e., soil moisture and climatological (soil temperature) conditions to assess the impact more holistically.

Barometric surface pressure was the only parameter that has demonstrated a positive and significantly high association with WNV outbreaks during and before an outbreak occurred. Research on this parameter is scarce. An eco-hydrologic model of the land surface-atmosphere interaction has been proposed by others as a means of estimating the role of atmospheric pressure on WNV outbreaks (Porporato et al., 2002; Entekhabi and Rodriguez-Iturbe, 1994) but mainly found soil moisture and temperature statistically significant. The vast majority of WNV related studies have concentrated on surface moisture rather than surface pressure to predict WNV disease dispersal dynamics in certain settings (Conley et al., 2014; Shaman, Day and Komar, 2010; Shaman, Day and Stieglitz, 2002; Shaman et al., 2002).

Volumetric soil water content, which covers soil texture, depth and the underlying groundwater level (European Centre for Medium-Range Weather Forecasts, 2018), was very significantly associated with disease prevalence in the best fitting model but

also associated with WNV disease outbreaks one month before the WNV outbreaks were recorded. However, the likelihood of this factor affecting disease distribution during the month of the reported WNV outbreak in humans was insignificant. Other studies also observed a similar pattern (Ukawuba and Shaman, 2018; Stilianakis et al., 2016). In Ukawuba and Shaman's study (2018), soil moisture outperformed other hydrological factors such as precipitation which probably stems for its ability to absorb surface water and thus promote and maintain larval development and mosquito reproduction.

Irrigation practices of agricultural crops and/or landscape maintenance has also been linked to increased human WNV disease (Eisen et al., 2010; Gates and Boston, 2009) as they can assist to the formation of breeding habitats, a natural attraction of avian and other wildlife, and trigger vector-host interaction and infection. Although irrigation systems and automated soil fertilisation mechanisms exist, human involvement in maintaining and monitoring those systems may lead to additional human exposure to breeding sites and contribute to elevated human WNV incidence rates. Reisen et al. (2004) suggested that high atmospheric pressure created a constant airflow pattern which led to WNV infections in a variety of locations in the States away from its Colorado epicentre. They concluded that a climate-driven mechanism contributed to this dispersion.

Based on the results of this study, soil moisture at larger spatial settings can be seen as an important hydrological factor; however, other underlying physical processes may have influenced these results. For example, soil moisture influences the apportioning of net radiation into underlying heat changes and as a result affects air temperature (e.g., Miralles et al., 2012) particularly during the warmer seasons of the year (Fischer et al., 2012; Lenderink et al., 2007). Other geological typologies and characteristics

such as soil and vegetation varieties, solar and long wave radiation level scan also play a role (Rodell, 2017; Shaman et al., 2002) which may present a limitation for the results of this study. For example, natural or induced increases in surface's ability to absorb light, namely albedo, may lead to changes in soil moisture and intensify vegetation in certain soil types (Lund et al., 2014; Eugster et al., 2000). Thus, if soil's innate ability to absorb water is co-examined with all these parameters it is more likely to deliver a more powerful estimation of disease prevalence.

Additionally, reduced soil water content in some locations, especially during the late spring and summer periods, may have an effect on WNV disease circulation directly effecting the entomological, avian, equid and human WNV cycle in locations where there is more water. A model that assesses and combines all key hydroclimatic water-related components -precipitation, soil moisture and humidity- can generate even more robust aetiologies for the observed increases in WNV occurrence in human populations. The present analysis showed that all three factors were strongly associated with disease circulation in the best fit model with variations across the other two models. This does not directly corollate with Stilianakis et al.'s observations (2016) that soil water content maybe be comparatively a better predictor than the other two hydroclimatic parameters.

Precipitation was also clearly associated with increased WNV human disease two months before the outbreak. Overall, existing research confirms that the role of precipitation is wide-ranging, complex and less consistent. Takeda et al. (2003) and Soverow et al. (2009) reported a similar pattern in the months preceding disease human outbreaks.

Although it can be argued that the volume of precipitation can influence disease prevalence, its role can vary as a result of ecological variations in vector activity (Paz and Semenza, 2013; Landesman et al., 2007; Moudy et al., 2007). For example, mosquito larval growth which requires standing water pools is naturally supported by heavy precipitation with Papa et al. (2010) opining that, although no significant association was found, it may have lent support to the proliferation of *Culex* species which led to the 2010 human WNV epidemic in Northern Greek and Italian regions. Landesman et al. (2007) and Nasci et al. (2001) also suggested that heavy rainfall may create those conditions that trigger increased mosquito activity to spawn human WNV epidemics. However, heavy rainfall may weaken the breeding nutrients required for larval development (Chevalier, Tran and Durand, 2013) or cause flooding of over ground or underground surfaces used by *Culex* larvae (Koenraadt and Harrington, 2008; Shaman et al., 2002) therefore limit their development which contributes to WNV spread.

On the other hand, low precipitation can lead to arid wetlands which can interrupt the usual breeding practices of some mosquito species (Chase and Knight, 2003; Shaman et al., 2002; Reisen, 1995). Chase and Knight (2003) found that drought can predict a future outbreak in the subsequent year within these populations. The organic materials produced in standing water during drought weather conditions may also become feeding attractions for avian hosts too (Paz et al. 2013). Thus, drought can also intensify the epizootic cycle between avian hosts and vectors as well as WNV infections within these groups (Shaman, Day and Stieglitz, 2005) which can consequently affect human populations. On the other hand, Hahn et al (2015) identified associations with both heavy and low precipitation; specifically, below the

average total precipitation influenced WNV infections in some US regions but higher than normal rainfall others.

As a result, it can be argued that total precipitation, either high or low, is subject to complex ecological reactions and conditions to arrive in a definite inference as regards its influence on WNV human disease. Although this study, alike others, demonstrated the likelihood of a close association between total precipitation and WNV human outbreaks in the best fit model and at the same month of an outbreak, it would be useful to test if the former's influence is linked or not to human outbreaks in the preceding year. There may be complex environmental, climatic and entomological interactions present that require further analysis to arrive to meaningful and absolute conclusions.

Relative humidity, alike precipitation, is also important for the mosquito life cycle (Panackal, 2016) and had a statistically relevant relationship with WNV human outbreaks in the studied countries. However, the present study found that maximum relative humidity is strongly and significantly negatively associated with human WN disease distribution in the preceding months, although not at the same time of an outbreak. This may be a result of (*Culex)* mosquitoes and/or human behaviours. Research on the relationship between this climatic factor and human disease outbreaks is limited. However, studies have shown that rises in surface humidity activates *Culex* mosquitoes feeding and egg production processes (Becker et al., 2010; Shaman and Day, 2007) especially during the early parts of spring providing larval and breeding habitats and a fertile environment that vectors and avian hosts can intensify interactions (Reisen et al., 2008; Stickman, 1988). In contrast, this study found that higher humidity produced lower human WNV infections. Others have shown

that humidity had only a negligible effect on mosquitoes' replication rates (Mulatti et al., 2014).

Other research has also reported significant positive correlations between relative humidity and hospital admissions in Israel (Paz, 2006), vector population intensification in the US (Walsh et al., 2008) and morbidity in humans in Europe and western Asia (Paz et al., 2013). Nevertheless, these studies discovered that ambient temperature was a stronger disease prognosticator than relative humidity in terms of WNV's incidence. This study, in contrast to the latter, identified that although both climatic factors were strongly and positively linked to disease outbreaks in the best fit model, relative humidity's impact was comparatively more significant on human WN disease outbreaks a month before an outbreak occurred than air temperature.

Stilianakis et al. (2016) found statistically significant associations between relative humidity, among other hydrological factors, and WNV infections in human populations compared to the results of this study which observed strong associations in the preceding months of an outbreak. This may be due to differences between one small regional climate and ecology and multiple diverse settings and climatic conditions. Although country wide results are more robust to make meaningful inferences, more research is needed at regional level to understand what influences such observations and whether they are representative and reliable across different regions. However, Groen et al (2017) also reported a correlation between very large lag but less so with shorter lags although this may be a result of collinearity; Hess, Davis and Wimberly (2018) showed that elevated late-season humidity was one of the most important predictors of WNV disease which does not necessarily correspond with the current findings that showed no statistically significant associations between WNV human

cases and relative humidity at the same month of the outbreak. Given that not all human WNV outbreaks studied took place in late season, it is not statistically appropriate to suggest that the two independent sets of results are contradictory. However, the present results are similar to previous findings, which predicted that low monthly humidity conditions in August were more significant in the best fitting model (Ukawuba and Shaman, 2018), when the WNV human outbreaks in Europe occurred in August.

There are also some limitations in this study in terms of other potential drivers which not accounted for in this approach. For example, human behaviour and activities (agricultural irrigation) (Eisen et al., 2014) during hot and humid seasons may also contribute to WNV disease spread and local outbreaks in humans but there is limited evidence to corroborate such hypothesis. Therefore, socioeconomics and behaviours require equally distinctive attention. Further, it would be equally important to analyse which humidity levels can affect WNV human disease incidence the most and how these vary in different ecologies in the respective countries experiencing epidemics. Although this research showed a clear association between relative humidity and increased number of human WNV cases, the precise influence of the values of low humidity in WNV human distribution in epidemics requires further exploration. The collective role of other hydrological conditions assessed here in a single model can provide evidence on how water related conditions including relative humidity affect WNV's epidemiology in the preceding months of an outbreak; however, further research on its interactions with and dependencies on other hydrological and meteorological factors such as air temperature in the preceding years can yield healthier conclusions.

Alike hydrological factors, wind is underrepresented in many studies examining the atmospheric factors affecting human WNV disease. Although some studies have indicated that WNV spread correlated with environmental factors such as wind, their emphasis has either been on low wind velocity (Stilianakis et al., 2016) or the influence on the vector-host interface (Reisen et al., 2004). Yasuoka and Richard (2007) reported negative correlations between wind with the presence of WNV but Stilianakis et al. (2016) found that low wind speed is positively associated during the same week of the human disease outbreaks. On the other hand, other research suggests that wind-blown vectors play a role in WNV distribution (Mackenzie, Gubler and Petersen, 2004) or a result of trends in bird migration (Ji-Guang and Mei, 1996) or storm concentration (Bengtsson, Hodges and Roeckner, 2006).

An area that has not been sufficiently researched is whether the direction of wind may be an important factor in predicting WNV human epidemics which this study sheds light on. In fact, wind patterns, both eastward and northward components of the 10-meter height wind, explored in this study showed a distinctive trend in terms of the direction of wind and its type of association with WNV outbreaks. Specifically, eastward wind with a vertical coordinate in height of 10m was found to be positively linked to human WNV outbreaks in the best fitting model of two months before an outbreak was reported. The most significant association across the three studied lags was observed during the same month followed by two months, with one month before having the least, although still significant, association with disease distribution. On the contrary, northward components of the 10-meter wind showed no significant association with WNV disease occurrence.

These wind parameters have not been previously explored in the context of WNV human epidemiology in Europe or elsewhere. Climate change may have played a role

in eastward wind components being more influential in disease spread with one other study reporting that strong winds had moved avian host species eastwards (Malkinson et al,2002) which may provide a partial rationale for these findings. What is worth postulating is that eastward winds are likely to be warmer than northward ones so this may justify the positive association uncovered by this study given that different forms of temperature have been found to correlate with disease outbreaks in human populations. Given the strong eastward wind association with disease outbreaks, one potential area to further explore is whether low or high eastward wind speeds maintain the same strong correlation in subsequent years at the same multicounty level. Further the more recent expansion of WNV human epi/endemics in traditionally colder EU countries such as Austria and Germany (ECDC, 2019) may be affected by eastward winds which requires further analysis. Overall, more research is needed on the role and causality of wind patterns on WN disease outbreaks, but the present results signify that wind direction can be an informative indicator of WNV infections in human populations.

6.1 Other data limitations

A number of limitations have been explored in this chapter including the ECDC data focusing primarily on clinical and diagnostic sources. Given that the vast majority of cases are believed to be asymptomatic (ECDC, 2019; Rossi et al., 2010) and therefore not reported, a future enhancement would be to make active surveillance compulsory in affected, endemic and epidemic areas. A randomised track and trace approach in those areas could help unearth undetected cases. This could potentially provide a true picture of human WNV incidence.

One further limitation of this study is that it did not employ an explicit spatio-temporal model (a model that accounts for autocorrelation in space and time) and instead applied a zero-inflated mixture model. Numerous spatial and spatio-temporal models have seen explored in various studies for data with excess zeros (Agarwal, Gelfand and Citron-Pousty, 2002) with epidemiological and public health studies utilising more than one framework. For example, Neelon et al. (2013) and Oleson and Wikle (2013) used spatio-temporal hurdle models to predict disease outbreaks whereas Musenge et al. (2013) and Amek et al (2011) applied zero-inflated models. Although the adopted zero-inflated model was less general compared to other models it is commonly used as it assumes that two different types of zeros may be present in the data (Arab, 2015).

6.2. Future questions

Based on the above review, it can be assumed that certain atmospheric and hydrological parameters, in particular air temperature, relative humidity, dewpoint temperature, surface pressure, total precipitation, eastward wind, and volumetric soil water contribute to the occurrence of WNV infections in southern and south-eastern two months prior to an outbreak occurring. In addition to the strengths and limitations of this review, a number of questions remain regarding the direct role of climate on WNV human epidemiology.

i. **Endemisation of WN disease.** The reappearance of human WNV outbreaks in continuous years and countries is potentially associated with the permanent establishment of the virus in certain geographical areas. The long-term predictive capacity of climate in human epidemiology requires continuous

monitoring to fully establish the most robust combination of the hydroclimatic parameters sustaining and reproducing WNV epidemics over time.

ii. **Overwintering dormancy (diapause) of infected mosquitoes**. The role of overwintering dormancy of *Culex pipiens* and some other mosquito species has been well researched (Zhou and Miesfed, 2009; Robich and Denlinger, 2005). Considerable evidence from the US suggests that overwintering is a direct contributor to human WNV outbreaks (Andreadis et al. 2010; Farajollahi et al. 2005; Bugbee and Forte 2004; Nasci et al. 2001). There is currently no data of how diapause, a period of delayed vector development in recurring periods of unfavourable climatological conditions, assists and/or affects the endemisation process of WNV infection in Europe. Assessing overwintering dormancy in different ecosystems and landscapes, such as urban and intermediate regions, could improve our WNV knowledge base, response and preparedness.

iii. **Genetic variation and climate.** In Europe, WNV human infections associated with Lineages 1 and 2 generally produce either asymptomatic, mild febrile, severe or fatal disease which demonstrates an important association between the genetic makeup of the virus or disease phenotype (Schuffenecker et al., 2005; Lanciotti et al., 2002). It is estimated that there are currently 7 lineages associated with human WNV infections worldwide. An understanding of WNV's genetic evolution and relevance to human WNV outbreaks in the European continent and more widely will further inform whether the above climatic conditions are direct predictors or indirect facilitators of genetic developments.

iv. **Climate change and other confounders.** The effects of global and continental warming on the diverse ecology of WNV in preceding years requires further investigation to fully establish whether longitudinal changes in meteorological conditions have accelerated future epidemics. Although certain climatic conditions have been found to be linked to human WNV outbreaks, targeted surveillance, improved awareness and enhanced monitoring may have contributed to increased reporting of human infections in the EU during 2010-2015 rather than being solely a result of viral intensification. However, it could also be hypothesised that the disease was continuously present in preceding years but remained underreported and undetected.

v. **Weather and environmental interfaces.** The role of ambient temperature, precipitation and humidity has preoccupied several studies (Manore et al., 2014; Harrigan et al., 2014); however, more research is needed on a variety of hydroclimatic factors which may, directly or indirectly, interact with the ability of hosts to migrate and vectors to adapt to new ecosystems. Kilpatrick et al. (2015) found that the long-term effects of land use alterations on ecosystems are greater than climatic conditions. The present review provided insights into multifactorial weather and landscape contributors but similar analysis in other continents may create a more robust evidence base. Other potential contributors such as vegetation type, solar and long wave radiation levels (Rodell, 2017; Shaman et al., 2002) may provide a stronger and deeper evidence bank.

vi. **Human interference.** A link between wind farming especially turbines (Osborn et al., 2000), irrigation practices of agricultural crops (Eisen et al., 2010; Gates and Boston, 2009) and human WNV incidence rates has been previously explored. Human ecological interference and their associated behavioural patterns may have negatively influenced the natural balance of a variety of ecosystems potentially enabling increased vector-host interactions and causing widespread infections. Certain climatological phenomena, i.e., droughts and storms, occupational practices i.e., farming, and/or socioeconomic challenges i.e., building housing near vector habitats may have jointly contributed to elevated numbers of human infections and therefore require further research. This would provide a better understanding as to whether unintentional interferences are potential triggers of human WNV infections.

vii. **Geographical and climate dependencies.** Initially West Nile Virus was circulating only in the temperate areas of Africa, Israel and India but has since moved to a number of diverse territories including Europe, parts of the US, Asia, Canada, and Australia. (ECDC, 2020; CDC, 2020; Calistri et al., 2010; Zeller and Schuffenecker, 2004; Murgue et al., 2001; Hubálek and Halouzka, 1999). Based on this review, most consecutive outbreaks in Europe occurred in southern and south-eastern Europe where the climate is warmer compared to Northern European countries. Information on climatic influences in South America and Africa is limited which would assist in better understanding WNV's performance in hotter climates. Further, autochthonous cases have been recently reported in traditionally colder EU countries such as Austria and Germany (ECDC, 2019). More research is therefore needed to understand

whether similar climatic influences are prevailing and/or if global warming is causing this geographical dispersion or widespread endemisation..

Overall, this study provided renewed and fresh evidence that numerous hydroclimatic factors can significantly affect human WNV distribution in countries experiencing concurring outbreaks as early as two months before the latter occurred. This is potentially a very important proof in terms of our understanding of the dynamics and mechanisms that trigger the re-emergence of WNV and potentially other vector-borne infectious diseases. Whether the climatic parameters impact was direct on the zoonotic transmission cycle of the pathogen or indirect, due to other underlying factors such as wider ecosystem adaptations, underlying physical processes and evolutional developments, requires further exploration. Crucially, the impacts of the changing climate on the vector-host interface may need to be adequately considered in any future assessment of WNV's transmission in the coming years.

CHAPTER 7- CONCLUSIONS

West Nile Virus continues to be an emerging public health risk and the need to clearly define the conditions affecting it as well as its long-term implications on human

populations is critical. A significant part of the world has environments and climatic conditions suitable for mosquitoes with population ageing considerably faster than in the past. These can lead to an estimated exposure of more than 2 billion people to WNV infections (Montgomery and Murray, 2015). Recent outbreaks in Europe have shown that many *Cx. pipiens* mosquitoes are infected with WNV (Martinet et al., 2019) which may be a reason for the endemisation of the virus in some European territories.

Numerous factors can lead to the geographical dispersion of the WV pathogen and vector in a variety of European ecosystems leading to significant outbreaks:

- This review presented population-level evidence for increased risk for patients >65 of age and men in Romania, Greece and Italy between 2010-2015. No specific therapeutic treatment or vaccine is available to treat human WNV infections and previous efforts have been paused due to insufficient information being available on targets groups. Further analysis on the health status of older age groups, behavioural traits in a variety of ecosystems and genetic dispositions would enrich these findings.

- Multiple climatic factors, air temperature, relative humidity, dewpoint temperature, surface pressure, total precipitation, eastward wind, and volumetric soil water, were found to have a significant impact on European WNV outbreaks in the best fitting model. The loglikelihood ratio was significant higher two months prior to an outbreak occurring. Their direct or indirect role on human WNV transmission requires further exploration. The impact of the changing climate, diapausing of infected mosquitoes, endemisation of WN disease and genetic evolution of the virus need to be considered in any assessment of human WNV transmission in the coming years. Several

confounding factors such as targeted surveillance, improved awareness, weather changes in previous years and enhanced monitoring may have contributed to the increase in reporting rather than indicate intensified viral presence.

- In addition to climatic factors, other drivers can play a key role is its distribution. Although country level environmental associations to human WNV outbreaks were identified, the overall picture was heterogenous. Environmental parameters utilised in this study, specifically the proximity to coastal, urban, rural and intermediate zones, have not been collectively explored elsewhere in countries experiencing consecutive human WNV epidemics. Therefore, direct comparisons with previous studies were not possible so more longitudinal and prospective data could enhance the evidence base generated through this review. The rise of infections in intermediate and urban areas may indicate that residential infrastructure is attracting larval multiplication. ECDC (2020) has recently suggested that the latter often occurs near man-made standing waters such as construction sites, road drains, water barrels and other types of exterior containers. The present results support this assumption. It is also assumed that the predominant landscape typology where most human population reside may be a factor in certain countries. A synthesised effort where demographic distribution per typology and WNV transmission is explored is necessary.

The need for multifactorial evidence on WNV to further inform public health actions, the development of therapeutics and other biological preparations for human use is therefore compelling.

CHAPTER 8- PUBLIC HEALTH IMPLICATIONS

The virus has the most prevalent geographical spread and the largest vector and host array compared to other mosquito-borne flaviviruses (Beck et al., 2013). WNV has gradually become endemic in all continents circulating in Africa, the Middle East, Southeast Asia regions, North America, Central America and Europe (Pauli et al., 2013). The present study's findings suggest that WNV public health policies may need to be adapted.

Burden of disease caused by co-circulation and co-infection

The impact and implications of co-circulation and possibility of co-infection of other seasonal febrile diseases on the public and healthcare systems need further exploration and may require renewed (multi) disease management planning especially for European countries with recurring endemic or epidemic outbreaks; this is particularly relevant as there are currently no licensed prophylactic vaccines available for the vast majority of these diseases including WNV. Funds for vaccine development for some of these diseases are being prioritised by the Coalition for Epidemic Preparedness and Innovations (CEPI), but given the WN pathogen's evolution, an investment into vaccine and therapeutics against the virus also requires adequate consideration and prioritisation.

Increasing public health promotion initiatives in certain demographic groups

The global human population is ageing significantly with individuals 60 and over expected to increase by over 2 billion by 2050 (United Nations, 2017) which can cause disproportionate use of healthcare provision. According to Yao and Montgomery (2016) older populations are more likely to contract infectious diseases with vaccination producing less protection and efficacious results compared to younger

populations. Age is a factor in, both natural and adaptive, immune reactions to infectious diseases, creating incongruous increases, reductions, and dysregulation in immune responses (Montgomery and Shaw, 2015). Mass cytometry and miRNA profiling as well as other technical approaches have been investigated to assess the link between age and host susceptibility to WNV infection and as a means of identifying potential therapeutic treatments (Yao and Montgomery, 2016). In addition to age, the vast majority of research shows that older men are more likely to develop neuroinvasive disease after WNV infection compared to women (ECDC, 2020; Brown et al*.,* 2007; Murphy et al*.,* 2005; Platonov et al*.*, 2001; Tsai et al*.*, 1998). Thus, a targeted public outreach campaign focusing on personal protection aimed at those demographic groups, i.e., older men, most likely to be as risk of WNV infection requires critical consideration to ensure that awareness is raised and minimise medical resourcing pressures and healthcare spending.

Enhancing monitoring and surveillance of WNV cases

In order to make meaningful inferences on WNV's true epidemiological profile, a better delineation and de-clustering of West Nile Fever and West Nile Neuroinvasive Disease cases may be required; this would allow better estimates of the true incidence and effect of disease on populations of affected geographic continental regions. Additionally, in a recent study by Gossner et al. (2017) inconsistent WNV surveillance practices in Europe were also observed and as a result more enhanced and standardised practices were recommended.

Risks assessments at European and country level will need to better capture reporting gaps and inconsistencies in epidemiological trends of countries experiencing continuous outbreaks; for example, a better understanding of the cause of a rapid

decline in cases compared to high incidence rate in preceding or following years (i.e., Greece in 2015). In addition to retrospective and in-year assessments, real-time monitoring and rapid information sharing of WNV distribution in its early stages can play a key role in informing and preparing clinicians and healthcare providers for future local, regional or national outbreaks. A centralised public health platform with information on live integrated surveillance, incidence in EU and neighbouring countries, guidance on personal protection, forms of clinical assessment and other critical areas would lend support to EU countries' public health containment efforts.

Utilising climatic and landscape intelligence to prevent disease outbreaks

In the absence of a human (WNV) vaccine and relevant therapeutics, prevention of WNV infection is addressed through vector control measures, use of mosquito repellents and suitable garments to reduce human infection (World Health Organization, 2017). Further investment in a comprehensive early warning prevention system which identifies climatic and epidemiological triggers could also have a positive impact on public health responses such as the provision of timely public advice regarding travel to infected areas, prospective blood donations from those areas (Commission Directive 2014/110/EU) and transmission risks for sensitive groups. Monthly summaries of the ECMWF's Copernicus Climate Change Programme, on average precipitation, surface air temperatures and other atmospheric parameters were notably higher than average in the WNF affected areas as early as March and/or May (Copernicus Climate Change Service, 2018). Various studies (Groen et al., 2017; Vogelset al., 2017; Tran et al., 2014; Paz et al., 2013) support the theory that certain climate conditions are associated with WNV transmission favouring an early upsurge of the vector population. Similarly, the present study identified that a number of climatic factors were a more significant predictor two months before an outbreak occurred

rather than the same month or a month earlier. Another study confirmed the link between climatic parameters and presence of WNV-RNA in *Culex pipiens* species as early as June (Kemenesi et al., 2014). Although weekly reports on WNV disease transmission are published by the ECDC, a synergetic approach with other relevant functions such as the European Centre for Medium-Range Weather Forecasts could further aid health protection and prevention.

Further, evidence on the type of landscape settings where outbreaks frequently occur could further inform public health planning. As per this study, certain typologies for example rural, urban, coastal and other areas can become valuable indicators for effective and timely public outreach campaigns. Such an approach could also positively impact not only of human health but minimise healthcare expenditure and secondary care pressures. Therefore, a predictive early warning model based on early climatic patterns and environmental conditions could provide a scientific response to local and national public health needs.

Calculating an expected outbreak size and diagnosing cases through algorithmic methods

Investment on methods of computationally calculating expected outbreaks and algorithmically diagnosing disease presence could further support public health responses at primary and secondary care level. A number of algorithmic models have been proposed to control the spread of infections and/or predictive future outbreaks (Green et al., 2008; James et al., 2007). Simulation techniques on a variety of types of populations have been explored in research to assess an outbreak's magnitude (Eames et al., 2015, Danon et al., 2011). Although most appear to be highly complex,

the need for disease specific, simple and reliable schemata to calculate potential outbreaks but also diagnose cases is becoming increasingly important.

In terms of diagnosis, an enhanced diagnostic approach aligned to the EU case definition could allow faster medical responses especially on cases demonstrating severe neurological symptomology such as inflammatory disease of the central nervous system. Given the commonalities in WNV symptomology with other circulating vector borne diseases, further development and employment of a testing algorithm for the detection of WNV virus would aid support in disease management. Such algorithms capable of serologically differentiating WNV from other selected viruses exist for poultry and horses (Yeh et al., 2012) but a standardised version for human populations requires further investment by European and other public health authorities.

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Annex A

Annex A. Most common arboviral diseases, their families and genera

Family Flaviviridae Genus Flavivirus Yellow Fever	Family Bunyaviridae Genus Orthobunyavirus California Encephalitis	Family Bunyaviridae Genus Phlebovirus Sandfly fever/Toscana virus	Family Flaviviridae Genus Flavivirus Murray Valley Encephalitis	Family Reoviridae Genus Coltivirus Colorado Tick Fever
Family Flaviviridae Genus Flavivirus St. Louis Encephalitis	Family Togaviridae Genus Alphavirus Eastern Equine Encephalitis	Family Togaviridae Genus Alphavirus Venezuelan Equine Encephalitis	Family Togaviridae Genus Alphavirus Mayaro Fever	Family Flaviviridae Genus Flavivirus Russian Spring Fever
Family Bunyaviridae Genus Orthobunyavirus LaCrosse Encephalitis	Family Togaviridae Genus Alphavirus Western Equine Encephalitis	Family Flaviviridae Genus Flavivirus Japanese Encephalitis	Family Bunyaviridae Genus Phlebovirus Rift Valley Fever	Family Flaviviridae Genus Flavivirus Usutu virus
Family Flaviviridae/ Genus Flavivirus West Nile Encephalitis	Family Flaviviridae Genus Flavivirus Powassan Encephalitis	Family Flaviviridae/ Genus Flavivirus Dengue	Family Togaviridae Genus Alphavirus Sinbis Virus Disease	Family Flaviviridae/ Genus Flavivirus Tick-borne encephalitis virus
Family Flaviviridae Genus Flavivirus Dengue hemorrhagic fever	Family Togaviridae Genus Alphavirus Chikungunya	Family Bunyaviridae Genus Orthobunyavirus Oropouche Fever	Family Flaviviridae Genus Flavivirus Kyasanur Forest Disease	Family Bunyaviridae Genus Orthobunyavirus Tahyna virus
Family Togaviridae Genus Alphavirus Ockelbo virus	Family Bunyaviridae Genus Nairovirus Congo-Crimean Hemorrhagic Fever	Family Togaviridae Genus Alphavirus Barmah Forest Fever	Family Flaviviridae/ Genus Flavivirus Louping-ill	Family Bunyaviridae genus Orthobunyavirus Inkoo virus

Source: Part of the list of diseases (excluding genera and families) came from FMEL, 2003; (website: http://eis.ifas.ufl.edu/sle/arbovirus.htm accessed 13.11.2014) p1; the genera and families were produced separately.

Annex B: Collinearity test

Variable1	Variable 2	Correlation
t2m1	t2m0	0.851477
t2m2	t2m0	0.532687
td2m0	t2m0	0.966152
td2m1	t2m0	0.786086
so2m0	t2m0	0.993062
so2m1	t2m0	0.838841
so2m2	t2m0	0.518559
t2m0	t2m1	0.851477
t2m2	t2m1	0.855516
td2m0	t2m1	0.866445
td2m1	t2m1	0.966977
td2m2	t2m1	0.791667
so2m0	t2m1	0.860305
so2m1	t2m1	0.993118
so2m2	t2m1	0.843033
t2m0	t2m2	0.532687
t2m1	t2m2	0.855516
td2m0	t2m2	0.592635
td2m1	t2m2	0.866201
td2m2	**t2m2**	**0.66666**
so2m0	t2m2	0.548795
so2m1	t2m2	0.864084

so2m2	**t2m2**	**0.993081**
t2m0	td2m0	0.966152
t2m1	td2m0	0.866445
t2m2	td2m0	0.592635
td2m1	td2m0	0.842061
td2m2	td2m0	0.538572
so2m0	td2m0	0.956795
so2m1	td2m0	0.851827
so2m2	td2m0	0.57824
t2m0	td2m1	0.786086
t2m1	td2m1	0.966977
t2m2	td2m1	0.866201
td2m0	td2m1	0.842061
td2m2	td2m1	0.843433
so2m0	td2m1	0.793736
so2m1	td2m1	0.957381
so2m2	td2m1	0.85137
t2m1	td2m2	0.791667
t2m2	**td2m2**	**0.66666**
td2m0	td2m2	0.538572
td2m1	td2m2	0.843433
so2m1	td2m2	0.798845
so2m2	**td2m2**	**0.956881**
t2m0	so2m0	0.993062

t2m1	so2m0	0.860305
t2m2	so2m0	0.548795
td2m0	so2m0	0.956795
td2m1	so2m0	0.793736
so2m1	so2m0	0.856438
so2m2	so2m0	0.539459
t2m0	so2m1	0.838841
t2m1	so2m1	0.993118
t2m2	so2m1	0.864084
td2m0	so2m1	0.851827
td2m1	so2m1	0.957381
td2m2	so2m1	0.798845
so2m0	so2m1	0.856438
so2m2	so2m1	0.860403
t2m0	so2m2	0.518559
t2m1	so2m2	0.843033
t2m2	**so2m2**	**0.993081**
td2m0	so2m2	0.57824
td2m1	so2m2	0.85137
td2m2	**so2m2**	**0.956881**
so2m0	so2m2	0.539459
so2m1	so2m2	0.860403
r2m1	r2m0	0.71738
r2m0	r2m1	0.71738

r2m2	r2m1	0.727012
r2m1	r2m2	0.727012
v2m1	v2m0	0.927617
v2m2	v2m0	0.843588
v2m0	v2m1	0.927617
v2m2	v2m1	0.931222
v2m0	v2m2	0.843588
v2m1	v2m2	0.931222
sp2m1	sp2m0	0.991157
sp2m2	sp2m0	0.989979
sp2m0	sp2m1	0.991157
sp2m2	sp2m1	0.991208
sp2m0	sp2m2	0.989979
sp2m1	sp2m2	0.991208
wv2m1	wv2m0	0.526682
wv2m0	wv2m1	0.526682
wv2m2	wv2m1	0.525093
wv2m1	wv2m2	0.525093
r2m0	t2m0	-0.6996
r2m1	t2m0	-0.72252
r2m2	t2m0	-0.56715
r2m1	t2m1	-0.70056
r2m2	t2m1	-0.71956
r2m2	t2m2	-0.70033

r2m1	td2m0	-0.62054
r2m2	td2m0	-0.55068
r2m2	td2m1	-0.6152
r2m0	so2m0	-0.69958
r2m1	so2m0	-0.72836
r2m2	so2m0	-0.57398
r2m1	so2m1	-0.70143
r2m2	so2m1	-0.72578
r2m2	so2m2	-0.70151
t2m0	r2m0	-0.6996
so2m0	r2m0	-0.69958
t2m0	r2m1	-0.72252
t2m1	r2m1	-0.70056
td2m0	r2m1	-0.62054
so2m0	r2m1	-0.72836
so2m1	r2m1	-0.70143
t2m0	r2m2	-0.56715
t2m1	r2m2	-0.71956
t2m2	r2m2	-0.70033
td2m0	r2m2	-0.55068
td2m1	r2m2	-0.6152
so2m0	r2m2	-0.57398
so2m1	r2m2	-0.72578
so2m2	r2m2	-0.70151