



## **UNIVERSITY OF TURIN**

Department of Medical Sciences

### **PhD in Medical Physiopathology**

XXXIII Cycle

*"Environmental, Meteorological  
and Climatic Determinants of  
Vector-Borne Diseases in Italy"*

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Academic Years: 2017/2018 – 2019/2020

Academic Field: MED/01

# CHAPTER 1

## Introduction

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### 1.1 Human health in a changing climate

Over the past decades, humankind experienced unprecedented improvements in terms of living conditions, such as increased food and water accessibility, better housing and hygiene. Improved living conditions, economic prosperity and scientific progress lead to an (unequal) increase in life expectancy worldwide<sup>1</sup>. However, economic development and human activities over the last centuries have caused multiple environmental changes, such as biodiversity loss, stratospheric ozone depletion, water and air pollution, soil contamination and, lastly, climate change<sup>2</sup>. As a consequence of the latter, in the last decades, the atmosphere and the oceans have warmed, the amount of snow and ice has diminished and the sea level has risen<sup>3</sup>. The anthropogenic emission of greenhouse gases (GHGs) since the industrial era has caused large atmospheric increases in the concentration of carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O). Experts of the Intergovernmental Panel on Climate Change (IPCC) have estimated a global mean surface temperature increase of 1°C from a pre-industrial baseline<sup>4</sup>. The magnitude of the projected rise in mean temperatures by the end of the 21<sup>st</sup> century compared to that of 20<sup>th</sup> century is substantially affected by different emissions scenarios, with predictions varying from 0.3°C to 4.8°C<sup>3</sup>.

Short term impacts of the global warming include the increase in frequency and intensity of extreme weather events such as heat waves, cyclones, floods, storms, heavy precipitation, and droughts<sup>5</sup>. In addition, different long-term changes in the environment occurred in the last decades, as both physical (e.g. glaciers, rivers and sea level) and biological system (e.g. terrestrial/marine ecosystem, crop production) are affected by human activities and climate change<sup>6,7</sup>. Not surprisingly, all these changes can have a huge impact on human health<sup>8</sup>.

The intuition about the close relationship between climate and health dates back to the ancient Greeks. Already Hippocrates underlined that climatic factors are fundamental in the explanation of health phenomena occurrence, as stated in his treatise "*Upon Air, Water and Places*"<sup>9</sup>, 400 BC:

*"Whoever wishes to investigate medicine properly, should proceed thus: in the first place to consider the seasons of the year, and what effects each of them produces because they are not at all alike, but differ much from themselves in regard to their changes. Then the winds, the hot and the cold, especially such as are common to all countries, and then such as are peculiar to each locality".*

Twenty-four centuries later, the knowledge about the relation between climatic factors and health has increased enormously. Our understanding of this relationship is changing how we consider the major determinants of human health. As underlined by the WHO<sup>10</sup>, while the health of individuals seems to relate mostly with behaviour, genetics, occupation and local environmental exposures, the health of populations is bounded to the health of the natural systems. Human population depends, indeed, on supplies of food and water, freedom from excess infectious diseases, and the physical safety conferred by climatic stability.

On the basis of the underling mechanisms, climate change impacts on human health can be classified in<sup>8,11</sup>:

1. Direct effects, which primarily relate to changes in the frequency of extreme weather, including heatwaves, drought, heavy rain and flooding;
2. Indirect effects, mediated through natural systems, such as changing activities of infectious disease vectors, occurrence of water-borne diseases, disruption of ecological systems and food yields;
3. Indirect effects, mediated by human systems, such as air pollution, mental stress, human conflicts and migration.

Direct impacts of climate change on human health, as heatwaves, have become a major focus in epidemiological research since 2003, when the northern hemisphere experienced one of the hottest summers of the modern history, causing thousands of deaths in Europe (with estimates ranging from 40,000 to more than 70,000 attributable deaths)<sup>12,13</sup>. Given their burden on public health, direct effects have been studied to a wide extent<sup>14</sup>. For instance, several epidemiological studies have shown that high air temperatures are strongly associated with adverse health outcomes, such as deaths, cardiovascular and respiratory events<sup>15-17</sup>. In addition, direct effects fit well with the epidemiological methods developed for investigating the role of a single exposure on health outcomes and the association between meteorological parameters and health outcomes is usually strong enough to permit inference about causes and effects<sup>18</sup>. On the contrary, indirect effects have been less investigated, given the complexity of the causal structure. Underlying mechanisms of indirect effects are characterized by complex interactions between causal components that act at different spatial and temporal scales<sup>19,20</sup>. Among the indirect effects, the impact of climate change on vector-borne diseases is one of the most studied<sup>14</sup>.

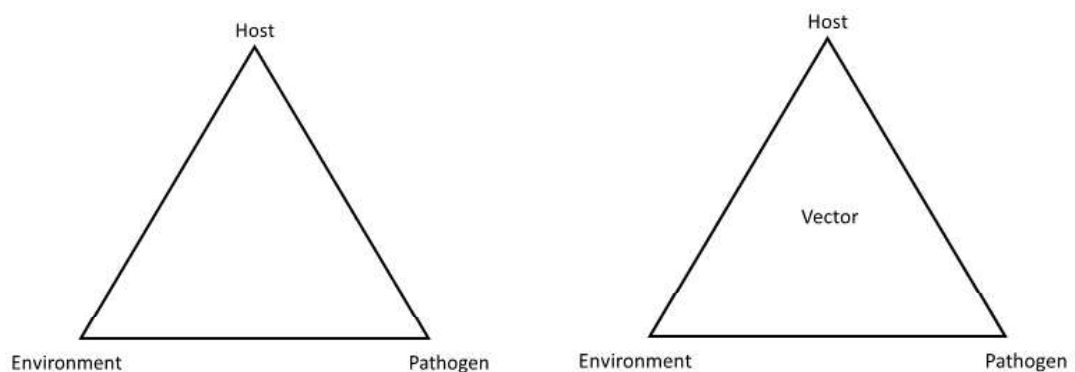
This research project was conceived to evaluate the potential effects of environmental, meteorological and climatic parameters on the spread of vector-borne diseases in Italy, paying specific attention to the potential effects of climate change.

## 1.2 Environment, climate and vector-borne diseases

Usually the causative nature of infectious diseases is described by the classic epidemiological triad. It consists of an equilateral triangle, whose vertices represent the host, the pathogen and the environment (Fig.1)<sup>21</sup>. Disease occurs when the three vertices connect; on the contrary, disrupting a connection using control measures, such as decreasing host exposure, will reduce pathogen transmission. However, the classical epidemiological triad must be modified to explain the complex biology of vector-borne diseases (Fig 1)<sup>21</sup>. The transmission of vector-borne diseases depends not only on the characteristics of the pathogen, host and environment, but also on the presence of a competent vector.

Given the multiple interactions between the environment-vector-pathogen-host, vector-borne diseases behave as dynamic systems that adapt and respond to external perturbations<sup>22</sup>. Potentially, any factor influencing the presence of the vector species can modify the likelihood of disease transmission. Consistently, it has been observed in the last decades that the ecology and transmission of vector-borne diseases can be potentially affected by anthropogenic environmental alterations, as deforestation, agriculture, urbanization, introduction of exotic species and climate change<sup>23–28</sup>.

Fig 1. Epidemiological Triads



Left panel: Classical epidemiological triad, Right Panel: Epidemiological triad adapted to vector-borne diseases.

Among others, it is recognized that climate change represent a leading factor in shaping the global distribution of vector-borne diseases<sup>29-36</sup>. Climate change can influence the vector-borne epidemiology by altering the meteorological and climatic parameters<sup>29-36</sup>. For instance, it is known that the air temperature impacts directly on the diseases transmitted by insect hosts, because arthropods are ectothermic organisms (namely, their blood temperature depends on environmental heat sources), and many infectious agents carried by them show proliferation rates that are particularly sensitive to temperature conditions<sup>37</sup>. Several mechanisms through which temperature may affect the risk of transmission of arthropod-borne diseases have been recognized, such as changes in the rate of vector population growth, vector feeding behavior, seasonality of vector activity, pathogen reproductive rates, and pathogen incubation period<sup>38</sup>. Also changes in precipitation patterns can impact on the ecology of vector-borne diseases<sup>8</sup>. However, the role of precipitation in the ecology of vector-borne diseases is more difficult to evaluate than that of temperatures, as they are often more complex and context specific. Concerning mosquitoes, during drought periods water pools become richer in organic materials that mosquitoes need to thrive, thus facilitating population growth of some species<sup>39</sup>. On the other side, heavy precipitation might lead to higher abundance of mosquitoes larvae and increase the potential for disease outbreaks in humans<sup>39</sup>. Concerning ticks, high precipitation levels during spring and summer can increase tick activity and survival rate, while dry periods can reduce the density of questing ticks<sup>40,41</sup>.

Lastly, long-term environmental changes driven by climate change can also affect natural habitats and human activities, which could further interact affecting the distribution and prevalence of vector-borne diseases<sup>42-44</sup>.

## 1.3 Vector-borne diseases in Europe

Since the beginning of 21<sup>st</sup> century, Europe has been experiencing unprecedented changes in the epidemiology of vector-borne disease<sup>42</sup>. It has been hypothesized that the interaction between globalization and climate change could explain, at least partially, the recent trends of these diseases observed in Europe. Globalization and international travels can favor the introduction of exotic pathogens and vectors in new areas<sup>45</sup>, while climate change can determine favorable meteorological conditions for the survival of the newly established pathogens and vectors and can also affect the geographical distribution of already endemic diseases<sup>46</sup>.

For example, confirmed outbreaks of autochthonous Chikungunya fever cases were recently reported in Italy and France<sup>47</sup>. Chikungunya Virus (CHIKV) is an alphavirus transmitted by *Aedes* mosquito species. In Europe, the risk of transmission of CHIKV is linked to the presence of *Aedes albopictus*, a highly invasive mosquito (Asian tiger mosquito). *A. albopictus* became established in Italy in 1990 and subsequently spread to neighboring countries, with a strong presence in the Mediterranean basin<sup>48,49</sup>. Although international travel played the main role in the introduction of the vector, it is likely that permissive climate offered favorable conditions for the spread of *Aedes albopictus*<sup>50</sup>.

With hundreds of cases recorded every year, West Nile Fever (WNV), a flavivirus transmitted by local mosquito species, as *Culex pipiens*, is now considered endemic in many European countries<sup>51</sup>. The WNV started circulating in the warmer regions of Europe in the late '90s after its introduction by migratory birds. WNV has caused seasonal outbreaks that have increased in frequency over the last years<sup>52</sup>. A massive outbreak occurred across southern and central Europe in 2018<sup>53</sup>. Different studies have reported that the magnitude of the seasonal viral circulation and human outbreaks are associated with high temperatures recorded during spring and summer<sup>39,51,54</sup>.

Concerning tick-borne diseases, the incidence of Lyme borreliosis is increasing in several European countries<sup>36</sup>. It has been documented that the presence of the *Ixodes ricinus*, the main vector for both Lyme borreliosis, is expanding both to higher latitudes (in Sweden)<sup>55</sup> and to higher altitudes (in Central Europe)<sup>56,57</sup>, probably as a consequence of increasing temperatures. Consistently, climatic models suggest that the abundance of *I. ricinus* might increase among northern European countries in the next years<sup>58,59</sup>.

It has also been observed that the geographical distribution of phlebotomine sand fly, the main vector for Leishmania parasites, is changing among European countries. Even though air temperatures play a relevant role in the survival and abundance of sand fly vectors, evidence of contribution of climate change on the Leishmaniasis distribution is still limited<sup>60</sup>. However, in Italy, climate change has been hypothesized as a potential factor for the observed northward shift of the disease<sup>61</sup>. Furthermore, several modelling studies have predicted that climate change could trigger the spread of sand fly vectors in areas outside their current geographical range, leading to an increased risk of leishmaniasis in several European countries<sup>62-64</sup>.

Climate change impacts on vector borne diseases are particularly relevant for Southern European countries, such as Italy. Southern Europe is characterized by mild winters and hot summers and is particularly susceptible to vector-borne diseases<sup>65</sup>. In addition, the mean temperatures of the Mediterranean basin have increased by 1.4 °C compared to preindustrial levels, a higher increase than the global mean warming of 1.0°C<sup>66</sup>. The warming trend observed in the last years in the Mediterranean area is characterized by longer and hotter summers, increased frequency of heat waves and changes in precipitation patterns<sup>67</sup>. All these changes driven by the global warming can directly impact the epidemiology of vector-borne disease in this area.



## 1.4 Aims and structure of the research project

This project aimed to evaluate the role of environmental, meteorological and climatic parameters on the epidemiology of vector borne diseases in Italy using different epidemiological approaches.

The general aims that motivated the research project were:

- a) Describing recent temporal trends and geographical distribution of vector borne diseases;
- b) Evaluating potential association between weather (temperature, humidity, precipitation) conditions and vector-borne diseases;
- c) Estimating the dose-response relationship and possible lag-effects of weather conditions on the incidence of vector borne diseases;
- d) Assessing the presence of circulating vectors in relation to environmental and climatic factors and observed human cases;
- e) Hypothesizing potential mechanisms by which meteorological factors under a climate change perspective might influence the ecology of vectors and the incidence of vector borne diseases.

To address these general objectives, the research project focused on two vector-borne pathogens: *West Nile Virus* and *Leishmania infantum*. Both pathogen under study are currently endemic in Italy, however they differ in terms of both historical presence and eco-epidemiology.

West Nile Virus is an arbovirus (*arthropod-borne virus*) that is maintained in nature through a zoonotic transmission between mosquitoes of the *Culex* genus (vector) and birds (hosts)<sup>68</sup>. The WNV can be accidentally transmitted to the humans after a bite of an infected mosquito. Human infection is symptomless in 80% of infected subjects, while 20% of cases develop a febrile syndrome, known as West Nile Fever (WNF). Fewer than 1% of infected subjects develop a neuro-invasive disease, characterized by meningitis or encephalitis symptoms<sup>69</sup>. In Italy, the first human case with a documented WNV infection was observed in 2008 in Emilia-Romagna<sup>70</sup>. Since then, the WNV circulation has been detected among vectors, birds and humans every year<sup>71</sup>. Italy was affected by a massive outbreak in 2018, when a 10-fold increase of cases with respect to the previous years was observed<sup>53</sup>. Recent changes in climatic conditions, particularly hotter spring/summer temperatures and abnormal rainfall amounts, might have led to an increasing number of human WNV infection cases in Italy. In addition, warmer winter may

have determined the survival of infected adult mosquitoes during winters (overwintering phenomenon) contributing to the persistence of the virus in the following years<sup>51,72,73</sup>.

The protozoan *Leishmania infantum* is one of the putative agents for the Visceral Leishmaniasis in the Old World and it is endemic in the Mediterranean Basin<sup>60</sup>. The transmission of the parasite is dependent on the presence of its natural vector (sand flies species) and reservoir hosts, such as infected domestic dogs<sup>60</sup>. The geographical distribution of phlebotomine sand flies is affected by several environmental parameters, such as altitude, land-cover, vegetation, air temperature and relative humidity<sup>74,75</sup>. For instance, air temperature is believed to influence both the vector distribution and the seasonal activity of the sand flies<sup>76,77</sup>. Cases of both canine and human leishmaniasis have been historically reported in central and southern peninsular Italian regions, characterized by dry and hot summers and mild winter temperatures<sup>78,79</sup>. However, in the last 20 years, both sand fly vectors and cases of canine and human visceral leishmaniasis have been observed in Northern Italian regions, traditionally classified as colder areas characterized by continental climate. The warming trend observed in the last decades has been suggested as the potential driver of the shift of the geographical distribution<sup>61</sup>.

Chapter 2, Chapter 3, and Chapter 4 of this thesis focus on the epidemiology of the *West Nile Virus*. Chapter 2 describes the temporal and spatial pattern of human West Nile Neuro-invasive Disease (WNND) observed between 2008 and 2017 and applies a spatio-temporal cluster detection method to identify high risk areas. Chapter 3 investigates the association between the human incidence of WNV infection and climatic parameters (air temperatures and precipitation) recorded in the weeks before the diagnosis using a time-stratified case-crossover study design. Chapter 4 describes the massive WNV outbreak observed in 2018, analyzing the relationship between air temperatures and precipitation with entomological parameters, namely the abundance of sampled *Culex* mosquitoes and prevalence of WNV infected *Culex* mosquitoes. Chapter 5 focuses on the geographical distribution of the Visceral Leishmaniasis in Piedmont, a newly endemic area in Northern Italy. Through a One-Health approach, it integrates environmental, entomological, animal and human data to map the risk of endemic transmission of *Leishmania infantum* in the study area.

## CHAPTER 2

# Recent rapid changes in the spatio-temporal distribution of West Nile Neuro-invasive Disease in Italy

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*Zoonoses Public Health.* 2020 Feb; **67**(1): 54-61

### Statement of Contributions of Joint Authorship

**Giovenale Moirano (PhD Candidate):** Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing - original draft

**Lorenzo Richiardi:** Methodology, Writing – review & editing

**Mattia Calzolari:** Methodology, Writing – review & editing

**Franco Merletti:** Methodology, Writing – review & editing

**Milena Maule (Supervisor):** Methodology, Supervision, Writing – review & editing

## ORIGINAL ARTICLE

# Recent rapid changes in the spatio-temporal distribution of West Nile Neuro-invasive Disease in Italy

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## Abstract

In Italy, the first human case of West Nile Virus (WNV) infection was reported in 2008 and, since then, the number of cases has been steadily increasing. In this study, we describe the temporal and spatial pattern of WNV infection risk among humans in Italy, focusing on the human cases of West Nile Neuro-invasive Disease (WNND) observed between 2008 and 2017. Incidence rates are estimated for each year and province under study. The incidence temporal trend is estimated using Poisson regression, and a spatio-temporal cluster detection analysis is performed to detect high-risk areas. In total, 231 WNND cases were notified in Italy between 2008 and 2017. The annual incidence rates increased during the study period (annual percentage change: 11.7%; 95%CI: -0.9%; 26.1%). A geographical spread of the disease was observed during the study period throughout Northern Italy, with an increasing number of affected provinces. Provinces close to the Po River (the main river in the north of Italy) and the Oristano province (in the Sardinia Island) experienced the highest incidence rates during the study period. Our study shows a gradual, but rapid spread of WNND across Northern Italy from east to west and suggests the hypothesis that provinces close to Po River might present ecological and climatic conditions favourable to the virus circulation.

## KEYWORDS

spatio-temporal pattern, West Nile Neuro-invasive Disease, West Nile Virus

## 1 | INTRODUCTION

West Nile Virus (WNV) is a vector-borne pathogen transmitted to humans by different species of *Culex* mosquitoes (Petersen, Brault, & Nasci, 2013). Human infection is asymptomatic in 80% of infected subjects, while 20% of cases develop a febrile syndrome, known as West Nile fever (WNF). Less than 1% of infected subjects develop a neuro-invasive disease, characterized by meningitis or encephalitis symptoms (Gyure, 2009). Infection of the central nervous system leads to West Nile Neuro-invasive Disease (WNND) that typically affects elderly subjects (at least 65 years of age) and immunocompromised patients (Davis et al., 2006).

WNV has an ecologically complex transmission cycle. The virus is maintained in nature through an enzootic cycle that involves birds and mosquitoes (Chancey, Grinev, Volkova, & Rios, 2015). Birds are considered the main reservoir of WNV, because they can develop a sufficiently high viraemia to cause the infection of a feeding mosquito (Pérez-Ramírez, Llorente, & Jiménez-Clavero, 2014). Ornithophilic mosquitoes are the main vectors, since they can transmit the virus from an infected bird to a susceptible one through salivary gland secretions. Humans and equids are dead-end host, because their viraemia is insufficient to infect mosquitoes.

Mosquitoes that feed on both birds (amplification hosts) and mammals (occasional, dead-end host) are referred to as bridge

vectors and are responsible for the human infection (Kramer, Styer, & Ebel, 2008). Specifically, *Culex pipiens* mosquitoes are considered the main bridge vectors of WNV in Italy (Vogels, Göertz, Pijlman, & Koenraadt, 2017). In Italy, the WNV transmission period overlaps with the activity period (May–November) of *Culex pipiens* (Bisanzio et al., 2011).

In Europe, until the mid-1990s, WNV infection cases were reported only sporadically. The first large outbreaks were observed in 1996 in Bucharest, Romania, with 393 WNND cases (Tsai, Popovici, Cernescu, Campbell, & Nedelcu, 1998) and in 1999 in Volgograd, Russia, with 318 WNND cases (Platonov et al., 2001). Since 2008, an increasing number of human cases of WNND have been notified in many countries of Eastern, Central and Southern Europe, with several outbreaks in Italy, Greece, Serbia, Hungary, Russia and Romania (Rizzoli et al., 2015).

Both biotic and abiotic factors are important drivers for the endemization of WNV observed in Europe in the last years (Paz & Semenza, 2013). Migratory birds are responsible for the introduction of the virus in new areas, while high densities of competent mosquitoes and the presence of susceptible local birds are essential for the amplification and the local transmission of the virus (Reisen, 2013; Rizzoli et al., 2015). Among abiotic factors, local environmental conditions and meteorological factors, including air temperature and precipitation, are relevant for the persistence of the virus (Paz & Semenza, 2013), for instance through their influence on the vector population dynamics.

Italy has been one of the most affected countries in Europe, reporting the first human case of WNV infection in 2008. In 2010, the Italian Ministry of Health implemented the first WNV surveillance plan at the national level, integrating veterinary, entomological and epidemiological data (Ministero della Salute, 2010). Different studies have documented that the integrated surveillance system was efficient in monitoring the spread of WNV and in detecting both symptomatic and asymptomatic human cases of WNV infection (Napoli et al., 2013; Rizzo et al., 2016, 2012). According to the national surveillance system, human cases have been reported in Italy every year since 2008 (Rizzo et al., 2016). Thus, WNV spread in Italy is considered an example of endemization of an emerging pathogen. The purpose of this work is to study the temporal and spatial patterns of WNND spread in Italy between 2008 and 2017 and identify spatio-temporal clusters.

## 2 | METHODS

### 2.1 | Study area

Italy is a densely inhabited country in Southern Europe with approximately 60 million inhabitants and a population density of 200 people per square kilometre. Population density is heterogeneous, and the Po Valley is the most densely populated area with almost half of the country population. Other densely packed areas include the metropolitan areas of Rome and Naples. Italy is administratively divided into 20 regions and 110 provinces. As the Italian Peninsula

### Impacts

- This study includes all cases of West Nile Neuro-invasive Disease (WNND) observed in the 10 years after the first appearance of a human case of WNND in Italy.
- West Nile Virus has spread rapidly in all Northern Italy, following an East–West geographical shift.
- The provinces close to the Po River, in Northern Italy, and the Oristano province, are identified as high-incidence areas.

extends over more than 1,000 km from the Alps in the north to the centre of Mediterranean Sea in the south, it presents a variety of climate systems ranging from continental climate of the inland northern areas (Po Valley) to the Mediterranean climate profile of coastal areas.

### 2.2 | Data collection

We restricted the analyses to the neuro-invasive form of the disease to avoid under-reporting in areas not covered by active surveillance. The number of human cases of WNND by province and year of diagnosis was obtained by the National Health Institute for the period 2008–2017. Information was collected examining published reports and periodic surveillance bulletins produced in the framework of the national surveillance plan (ISS, 2018; Rizzo et al., 2012, 2009). For each study year, data of the Italian population and the population of each of the Italian provinces were obtained from the Italian National Statistical Institute (ISTAT) (Demo-Geodemo, 2018).

### 2.3 | Statistical analysis and results representation

We first analysed the annual incidence rates of WNND cases notified in Italy between 2008 and 2017. Temporal trends were estimated using Poisson regression. Incidence rates were represented graphically through choropleth maps at the province level.

To identify spatio-temporal pattern of human WNND cases, we analysed the area-specific incidence rates for each year under study (2008–2017) applying a model-based cluster detection method for areal data (Gomez-Rubio, Serrano, & Rowlingson, 2018). This method tests and selects the most relevant spatio-temporal clusters on the basis of the likelihood of different Poisson regression models including potential clusters with different spatial and temporal dimensions as covariates.

Finally, we focused on the geographical distribution of WNND cases diagnosed in Northern Italy, in order to assess the presence of a geographical shift of cases from east to west during the study period. We split the study period into two 5-year intervals (2008–2012 and 2013–2017) and computed, for each of the two, the cumulative distribution curves of WNND cases as a function of the

**TABLE 1** Number of WNND cases by province of diagnosis and corresponding incidence rates per 1,000,000 person-years in the period 2008–2017

Region	Province	Number of WNND cases	WNND Incidence Rate (per million)
Emilia-Romagna		82 (35.5%)	
	Modena	23	3.28
	Reggio Emilia	12	2.25
	Bologna	20	2.00
	Ferrara	16	4.51
	Parma	5	1.13
	Ravenna	1	0.25
	Piacenza	4	1.39
	Rimini	1	0.30
Veneto		69 (29.9%)	
	Venezia	24	2.80
	Rovigo	17	6.97
	Treviso	19	2.14
	Verona	3	0.33
	Vicenza	4	0.46
	Padova	1	0.11
	Belluno	1	0.48
Lombardia		55 (23.8%)	
	Mantova	19	4.58
	Cremona	9	2.49
	Milano	6	0.19
	Pavia	10	1.82
	Lodi	8	3.49
	Brescia	3	0.23
Friuli Venezia Giulia		6 (2.6%)	
	Udine	3	0.56
	Pordenone	2	0.63
	Gorizia	1	0.71
Piemonte		4 (1.7%)	
	Asti	2	0.91
	Torino	1	0.04
	Novara	1	0.27
Toscana		2 (0.8%)	
	Livorno	2	0.59
Sardegna		10 (4.3%)	
	Oristano	9	5.50
	Olbia-Tempio	1	0.63
Puglia		1 (0.4%)	
	Foggia	1	0.16
Basilicata		1 (0.4%)	
	Matera	1	0.50
Sicilia		1 (0.4%)	
	Trapani	1	0.23

longitude of the capital of the province in which the diagnosis of the case was posed.

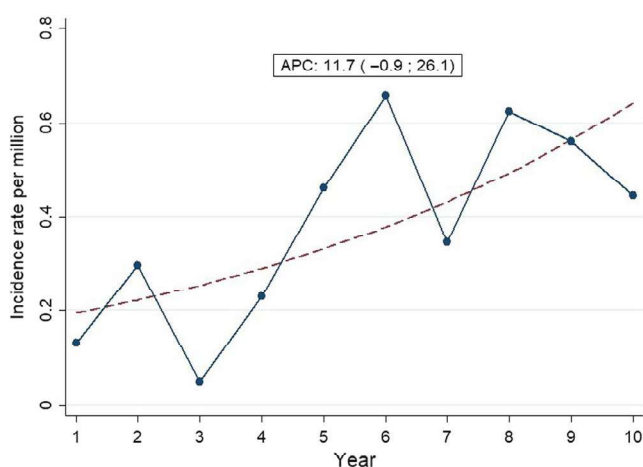
### 3 | RESULTS

In total, 231 WNND cases were notified in Italy between 2008 and 2017. The total number of cases and the corresponding incidence rates by provinces are reported in Table 1. We observed a positive trend in incidence with an estimated annual percentage change of 11.7% (95%CI: -0.9%; 26.1%) (Figure 1).

As shown in the maps of the annual incidence rates (Figure 2), the number of provinces affected increased from 2008 to 2017. Between 2008 and 2012, the rates were low and only a small number of provinces in the north of Italy were affected. In 2013, the rates and the number of affected provinces started to increase. Overall, Northern Italy reported WNDD cases every year of the study period, while sporadic cases appeared in Central and Southern Italy.

Spatio-temporal clustering analysis of the entire 10 years identified a total of four high-risk areas, denoted with letters from A to D and shown in Figure 3. Cluster A was present in 2008 and 2009 and included only two provinces in North-East of Italy. In 2011, two new clusters appeared: a large cluster in the North-East of the country, which lasted for three years (Cluster B), and a small but persistent one in the province of Oristano, which remained present throughout the study period (Cluster C). The largest one (Cluster D) included 14 provinces close to the Po River in Northern Italy and covered 5 years (2013–2017). Cluster D was shifted towards west in comparison with the two previous clusters (Clusters A and B).

The cumulative distribution curves of WNND cases by longitude showed an East–West geographical shift from 2008–2012 to 2013–2017. Figure 4 shows that, cumulating case from west to east, in the second period, 50% of cases were accrued approximately 110 km



**FIGURE 1** Italian WNND incidence rate (per 1,000,000 person-years) in the period 2008–2017 and estimated time trend (dashed line); APC: annual percentage change (%)

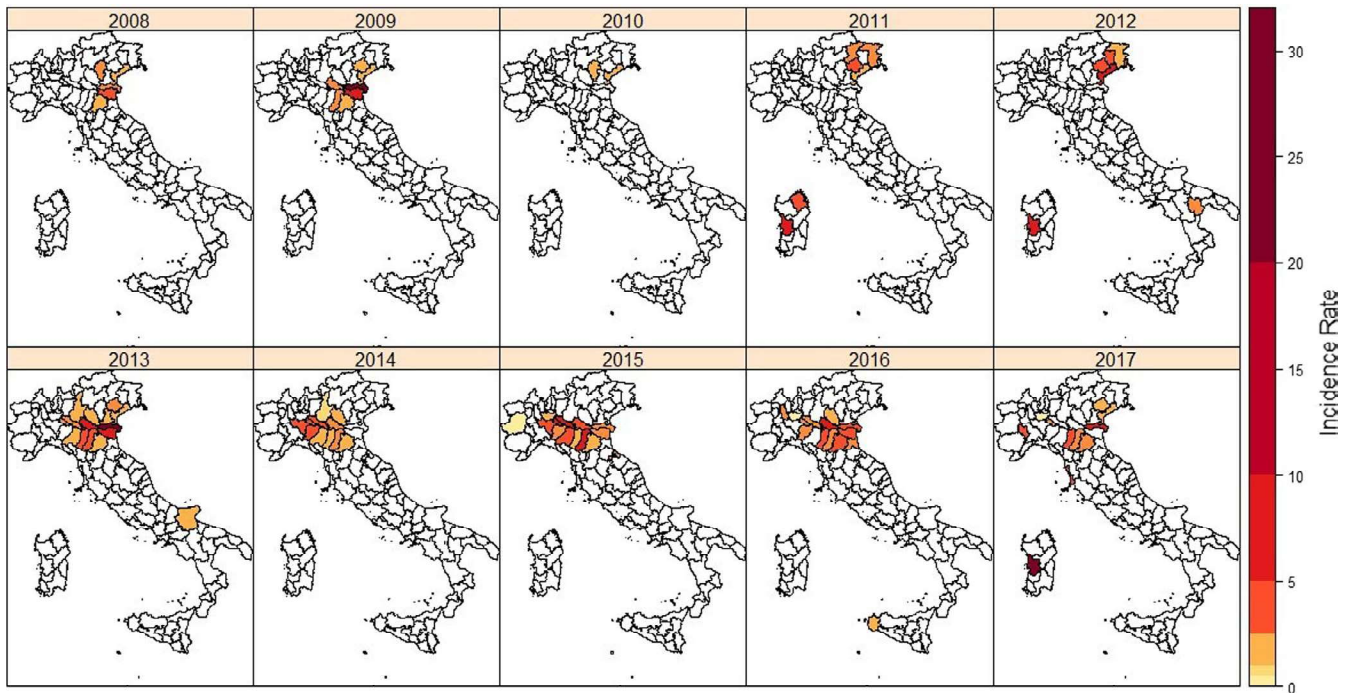
west of the point where the same percentage of cases were accrued in the first period.

### 4 | DISCUSSION

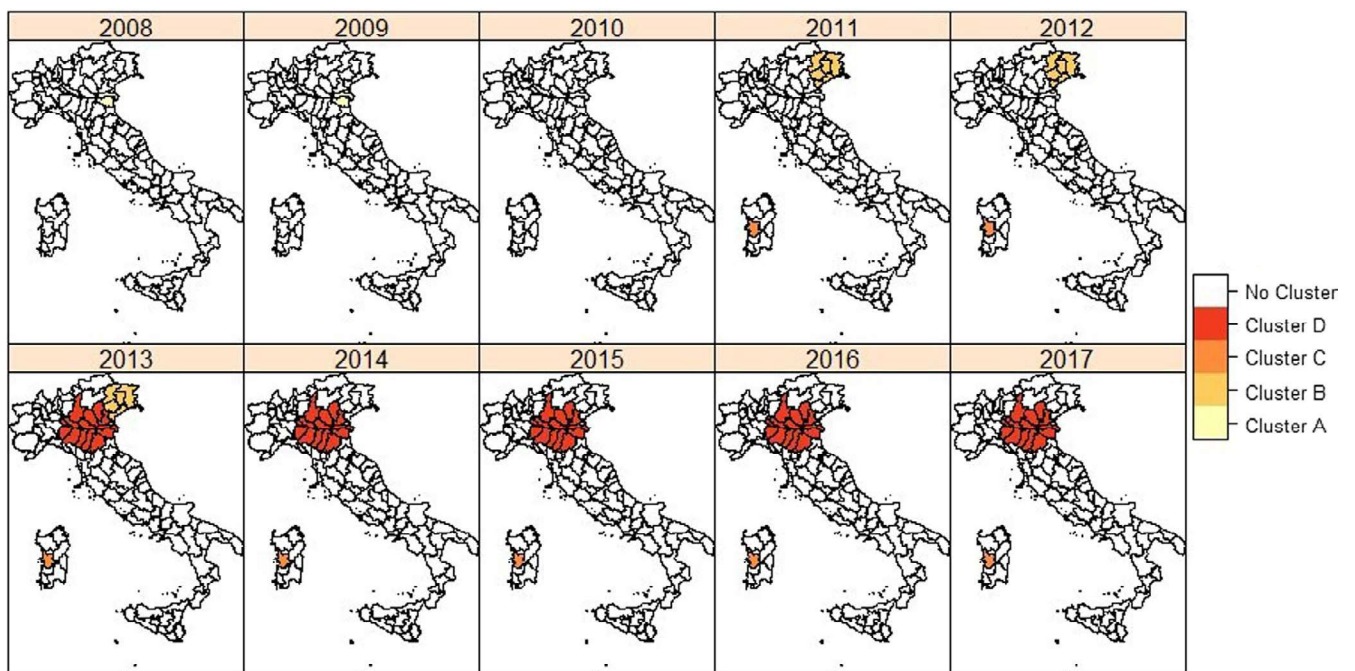
This paper describes the spatio-temporal distribution of human WNND cases observed in Italy in the period 2008–2017. The key features are the constant increase in incidence and a geographical shift from east to west, mainly in the provinces along the Po River. Given that in Italy the first human case of WNV infection was observed in 2008, and Italy is now one of the most affected countries in the European Union, we can conclude that Northern Italy is currently facing the process of endemization of the WNV.

Once introduced in new areas by infected migratory birds, WNV must establish an endemic cycle of transmission among immunologically naïve birds and mosquitoes (Chancey et al., 2015). It has been shown that the virus can circulate among reservoirs and vectors for several years before causing significant human outbreaks (Zehender et al., 2017). It has been hypothesized that the persistence of the virus in new affected areas might be due to infected birds that develop persistent WNV infection. Birds with chronic infection act like reservoirs and can promote WNV circulation in the subsequent transmission seasons (Komar et al., 2003). In addition, survival of infected adult mosquitoes during winters (overwintering phenomenon) or vertical transmission to eggs might contribute to the persistence of the virus in the following years (Rudolf et al., 2017). Possibly, the two ways of overwintering can have different relevance in different geographical area, characterized by different ecological condition. If the virus persists for subsequent years in the enzootic cycle, the prevalence of virus within host and vector populations will gradually increase, leading to higher risk of human infection over time. These hypotheses seem to be confirmed also by the results obtained over the years by the Italian integrated surveillance system. In Italy, with few exceptions, mosquitoes and wild birds tested positive well before the appearance of human and horse cases (Bellini et al., 2014) suggesting that the virus circulated and amplified in several species of wild birds and *Culex pipiens* mosquitoes, before spreading and causing cases in dead-end hosts such as humans and equids (Calzolari et al., 2013).

The epidemic pattern of WNND human cases recorded in Italy might reflect two subsequent distinct WNV introductions. Circulation between 2008 and 2012 was sustained by lineage 1 strains. After 2013, recorded cases were caused by a lineage 2 strain, affine to the virus already circulating in Europe and first detected in Hungary in 2004 (Barzon et al., 2013). Interestingly, this strain had already been detected in mosquitoes and one bird in a North-Eastern Italian region (Friuli Venezia Giulia) in 2011 (Savini et al., 2012), highlighting the possibility of silent circulation of the virus before the appearance of a human outbreak. Moreover, a different strain, affine to the Volgograd lineage 2, was detected in 2014 (Ravagnan et al., 2015), suggesting that



**FIGURE 2** WNDD incidence rates (per million person-years) in Italian provinces by year of diagnosis



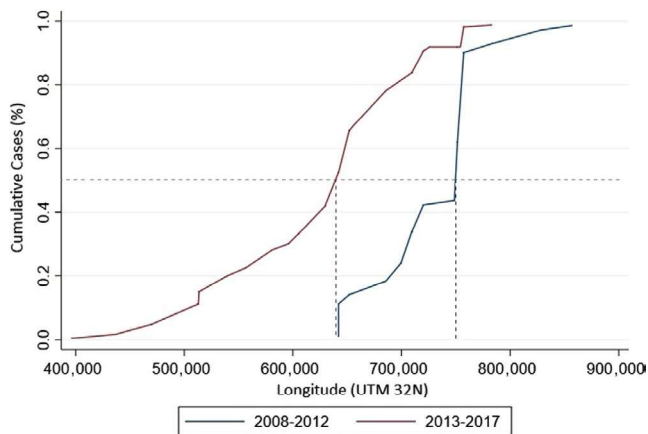
**FIGURE 3** Spatio-temporal clusters for the period 2008–2017 in Italy. Cluster A: Ferrara and Rovigo provinces ( $n = 2$ ) in 2008–2009; Cluster B: Belluno, Gorizia, Pordenone, Treviso, Udine and Venezia provinces ( $n = 6$ ) in 2011–2013, Cluster C Oristano province ( $n = 1$ ) in 2011–2017; Cluster D: Bologna, Bergamo, Brescia, Cremona, Ferrara, Lodi, Mantova, Modena, Parma, Piacenza, Reggio Emilia, Rovigo, Pordenone and Verona provinces ( $n = 14$ ) in 2013–2017

several introductions of different WNV strains have likely occurred in Italy at different times.

During the study period, we observed an East–West geographical shift of human WNND cases in Northern Italy. In the first half of the study period (2008–2012), the two identified clustering

areas were located in the North–East of Italy, while in the second half of the study period (2013–2017) the main clustering area was observed among the central provinces of Northern Italy. These results were confirmed by the East–West geographical shift observed when plotting the cumulative distribution curves of WNND cases





**FIGURE 4** Cumulative distribution curves of WNND cases diagnosed in Northern Italy by longitude of the Capital of the province of diagnosis for period 2008–2012 and period 2013–2017. The dotted lines represent the 50th percentile of the distributions of cases and the corresponding longitude at which 50% of the cases were accrued (starting from East). The left shift of the more recent period shows a spread towards West of incident cases. For example, in 2013–2017, 50% of cases were accrued at approximately 110 km West of the point where 50% of cases were accrued in 2008–2012

by longitude. Interestingly, similar results have been found by a phylogenetic analysis conducted among humans, mosquitoes and birds, which underlined that the WNV spatial spread observed in Northern Italy in recent years probably occurred along the Po river and its main tributaries with an East–West gradient (Zehender et al., 2017). These results suggest that Po River and its neighbouring areas might present environmental and ecological conditions that can promote WNV transmission and circulation. Among the others, main drivers in Northern Italy might be hot and humid summers, presence of wetlands and irrigated croplands, highly fragmented natural sites, local bird abundance and high density of *Culex* mosquitoes (Marcantonio et al., 2015; Paz & Semenza, 2013; Tran et al., 2014). All these factors can interact providing ideal conditions for establishing endemic cycles of WNV and increasing the probability of human infection.

Anomalies in climatic and meteorological conditions observed in recent years might have played a role in the geographical spread of WNV in Northern Italy and might partially explain the spatial pattern of WNND human cases. It has been hypothesized that meteorological anomalies during spring/summer might affect the seasonality of the disease by anticipating the starting time of the transmission period and favour an early increase in the number of infective mosquitoes (Paz, 2015). Interestingly in 2013, the year with the highest number of WNND cases during the study period was characterized by extraordinarily hot summer temperatures and areas where both infected humans and mosquitoes were observed were characterized by higher summer temperatures, compared to the areas without evidence of viral circulation (Calzolari et al., 2015). It has also been shown that periods characterized by high temperatures and above than average rainfall can affect incidence of human

WNV infection cases in the following weeks (Moirano et al., 2018; Paz et al., 2013).

Among Central and Southern regions, the Oristano province, in the Sardinia region, was detected as a spatio-temporal cluster. Interestingly, this area is rich in natural wetlands and lies in the western routes of migratory birds that are well-known risk factors for virus circulation (Paz & Semenza, 2013). Thus, the Oristano province is likely to be a suitable area for the interaction of migratory birds, resident birds, competent mosquito vectors and humans.

The main limitation of our study is due to the differential surveillance system that rules in endemic areas and not endemic areas. National surveillance plans are defined as endemic area provinces in which the presence of WNV has been identified among birds, mosquitoes or mammals (equids or humans). Since 2010, endemic areas (mainly regions of Northern Italy) have implemented active surveillance based on the research of WNV antibodies among both hospitalized cases of aseptic meningitis/encephalitis and cases of fever with rash and have organized a mandatory screening of all blood donors. On the contrary, non-endemic areas report human cases according to a passive notification system (ISS, 2018). Thus, it is plausible that part of the spatial heterogeneity of WNV cases might be explained by a greater ease of diagnosis in endemic areas. However, we included in the study only WNND cases, excluding asymptomatic blood donors and fevers with rash. WNND cases develop neurological symptoms and are more likely to receive aetiological diagnostic tests also in not endemic areas and, therefore, less likely to be underdiagnosed, even though it cannot be excluded that some WNND cases have been missed by the surveillance system in not endemic areas.

The main strength of our study is that it includes all WNND cases diagnosed in Italy since the first appearance of a human case of WNV infection. To our knowledge, this is the first study attempting to describe systematically the epidemiological patterns of WNND cases in Italy since its first appearance in humans and for a 10-year period (2008–2017). Our results may lay the foundations for future studies aimed at investigating the potential mechanisms underlying WNV outbreaks, such as the recent outbreak observed in 2018 in Italy (230 cases of WNND reported by ISS) (ISS, 2018).

In conclusion, our study shows a gradual, but rapid, geographical spread of WNND across Northern Italian provinces. This suggests that WNV has the capacity to spread into areas not previously interested by the viral circulation and can persist in areas already affected. Disease incidence exhibited geographic clustering, suggesting that provinces close to Po River experience higher risk and might present ecological and climatic conditions favourable to virus circulation. Further studies focused on the complex interaction between biotic and abiotic factors are needed to identify major determinants of spatio-temporal variability in WNV incidence, useful to predict areas and periods at risk of WNV outbreaks.

## CONFLICT OF INTEREST

Nothing to declare.

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**How to cite this article:** Moirano G, Richiardi L, Calzolari M, Merletti F, Maule M. Recent rapid changes in the spatio-temporal distribution of West Nile Neuro-invasive Disease in Italy. *Zoonoses Public Health*. 2019;00:1–8. <https://doi.org/10.1111/zph.12654>

# CHAPTER 3

## West Nile Virus infection in Northern Italy: Case-crossover study on the short-term effect of climatic parameters

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*Environ Res.* 2018 Nov; **167**:544-549.

### Statement of Contributions of Joint Authorship

**Giovenale Moirano (PhD Candidate):** Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft

**Antonio Gasparri:** Methodology, Writing – review & editing

**Fiorella Acquaotta:** Data curation, Writing – review & editing

**Simona Fratianni:** Data curation, Writing – review & editing

**Franco Merletti:** Methodology, Writing – review & editing

**Milena Maule (Supervisor):** Methodology, Supervision, Writing – review & editing

**Lorenzo Richiardi:** Methodology, Supervision, Writing – review & editing



## West Nile Virus infection in Northern Italy: Case-crossover study on the short-term effect of climatic parameters

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### ARTICLE INFO

#### Keywords:

West Nile Virus  
Temperatures  
Precipitations  
Lag-distributed models  
Case-crossover

### ABSTRACT

**Background:** Changes in climatic conditions are hypothesized to play a role in the increasing number of West Nile Virus (WNV) outbreaks observed in Europe in recent years.

**Objectives:** We aimed to investigate the association between WNV infection and climatic parameters recorded in the 8 weeks before the diagnosis in Northern Italy.

**Methods:** We collected epidemiological data about new infected cases for the period 2010–2015 from the European Center for Disease Control and Prevention (ECDC) and meteorological data from 25 stations throughout the study area. Analyses were performed using a conditional Poisson regression with a time-stratified case-crossover design, specifically modified to account for seasonal variations. Exposures included weekly average of maximum temperatures, weekly average of mean temperatures, weekly average of minimum temperatures and weekly total precipitation.

**Results:** We found an association between incidence of WNV infection and temperatures recorded 5–6 weeks before diagnosis (Incidence Rate Ratio (IRR) for 1 °C increase in maximum temperatures at lag 6: 1.11; 95% CI 1.01–1.20). Increased weekly total precipitation, recorded 1–4 weeks before diagnosis, were associated with higher incidence of WNV infection, particularly for precipitation recorded 2 weeks before diagnosis (IRR for 5 mm increase of cumulative precipitation at lag 2: 1.16; 95% CI 1.08–1.25).

**Conclusions:** Increased precipitation and temperatures might have a lagged direct effect on the incidence of WNV infection. Climatic parameters may be useful for detecting areas and periods of the year potentially characterized by a higher incidence of WNV infection.

### 1. Introduction

West Nile Virus (WNV) is a globally distributed RNA virus of *Flaviviridae* family (Campbell et al., 2002). It is maintained in nature through an enzootic cycle. Adult mosquitoes, generally of *Culex* genus, represent primary bridge vectors, while susceptible bird species play the role of amplification hosts (Chancey et al., 2015). Humans usually develop infection after being bitten by an infected mosquito. Infection in humans is generally asymptomatic, but 20% of infected subjects can develop a febrile syndrome, known as West Nile Fever (WNF), and less than 1% of infected subjects can develop a West Nile Neuroinvasive Disease (WNND) characterized by encephalitis or meningitis symptoms (David and Abraham, 2016).

In recent years, several outbreaks of WNV infection have been recorded in many European and Mediterranean countries (Rizzoli et al., 2015). Infected migratory birds are responsible for the introduction of the virus in new areas, while native mosquitoes feeding behaviour, presence of susceptible endemic birds and local environmental conditions are essential for persistence and amplification of the virus in new areas (Rizzoli et al., 2015). Climatic and meteorological conditions have been suggested as important factors for virus transmission in newly affected areas (Paz, 2015; Paz et al., 2013). High extrinsic temperatures are associated with virus replication and the growth rate of the vector population (Gubler et al., 2001). Levels of precipitation are also believed to play an important role in pathogen/vector ecology: some studies reported that vector replication and activity are positively

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<https://doi.org/10.1016/j.envres.2018.08.016>

Received 24 April 2018; Received in revised form 3 July 2018; Accepted 10 August 2018

Available online 18 August 2018

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associated with heavy rainfall and other studies reported that mosquitoes' abundance is associated with drought periods (Nile et al., 2009; Paz, 2015).

In Italy, the WNV was isolated for the first time in 1998 in 14 equine cases and the first human case was identified in 2008. Since then, human cases of WNV infection have been repeatedly notified, and now the virus is considered endemic in Italy (Rizzo et al., 2016). Concurrently the number of provinces set in Northern Italy affected by WNV circulation has increased during the study period (3 provinces in 2010 vs 16 in 2015). Thus, Italy can be considered as an example of area that is facing the process of endemization of an emerging pathogen.

The purpose of this study is to evaluate the short-term effects of air temperatures and precipitation on the incidence of WNV infection to understand the role of climatic parameters in the spread of WNV infection in an area, such as Northern Italy, where the process of endemization has recently started.

## 2. Methods

### 2.1. Data collection and elaboration

Epidemiological data were obtained from the European Center for Disease Control and Prevention (ECDC). In our study, WNV cases are subjects resident in Northern Italy who, during the period 2010–2015, met the European criteria for probable or confirmed case of WNV infection (European Commission Decision 2008/426/E). Cases are confirmed if at least one following laboratory criterion is present: isolation of WNV from blood or Cerebrospinal Fluid (CSF), detection of WNV nucleic acid in blood or CSF, WNV specific IgM in CSF, WNV IgM high titer and subsequent detection of WNV IgG. Cases are considered probable in presence of stable and elevated virus specific serum antibody titer in association with one clinical criterion (fever, meningitis or encephalitis) or evidence of an epidemiological link that proves animal/human to human transmission. Thus, notified cases recorded by ECDC are a heterogeneous population and include: WNV positive blood donors, cases of WNF and cases of WNND. For each case, the ECDC provides information on the year, the week and the geographical province of diagnosis.

Meteorological data were obtained from the Regional Environmental Protection Agency (ARPA) for each province that reported at least one case of infection between 2010 and 2015. We used the information recorded by the land-based meteorological stations set in the capital of each province. Meteorological data included minimum, mean, maximum daily temperatures, and daily precipitation. On the daily data of temperatures and precipitation a quality control was carried out to exclude the possibility of measurement error (Fortin et al., 2017; Acquaotta et al., 2016; Zandonadi et al., 2016). In order to conform meteorological data to epidemiological data, we calculated the weekly average of the minimum, mean and maximum temperatures, as well as, the weekly total precipitation. We considered missing all weeks with at least one missing daily information (information missing on weekly scale: 4.4% for maximum temperatures, 6.4% for mean temperatures, 5.1% for minimum temperatures and 6.1% for total precipitation).

### 2.2. Study design

To estimate the association between climatic parameters and WNV infection, we used a case-crossover design, which is a special case-control design where every case serves as its own control and originally developed to study the acute effect of transient exposures on the risk of rapid onset events (Maclure and Mittleman, 2000). For each case, exposures occurring during the period prior to the event (known as “hazard period”) are compared to exposures at comparable control periods (known as “reference periods”) (Janes et al., 2005; Levy et al., 2001). In

our study, control periods were identified according to a time-stratified sampling scheme, which uses fixed and relatively short time strata (e.g. calendar month) to match case and control periods (e.g. calendar week). Time-stratified case-crossover design has been repeatedly applied in environmental studies as it can control for long time trends (e.g. variability from year to year) and seasonality (variability from month to month) and can provide results equivalent to time series regression (Bateson and Schwartz, 1999; Navidi, 1998; Lu and Zeger, 2007). We further modified the original time-stratified approach with the inclusion of a b-spline function of time to control for residual temporal variation within strata, given the strong seasonality of WNV infection (Whitaker et al., 2007).

After observing the 2010–2015 cumulative epidemic curve, we firstly defined the transmission period of WNV, identifying the time interval going from the 27th to the 46th weeks of each year (length of 20 weeks). We secondly divided the identified period into 5 strata, each of 4 weeks length. For each week in which at least one human WNV case was reported (case period), we selected the other 3 weeks of the stratum as control periods. Exposure to meteorological variables, recorded in the capital of the province, were attributed to each case on the basis of the province in which her/his diagnosis was made.

### 2.3. Statistical analysis

The analysis was performed using conditional Poisson regression (Armstrong et al., 2014). Since weather effects on infectious disease risk may be delayed (lag-effect), we studied the incidence of WNV infection in relation to meteorological data recorded during the 8 weeks prior to the diagnosis. Therefore, we implemented a conditional Poisson regression in the context of lag-distributed models, which are suitable to explore the delayed effect of an exposure. Specifically, we used distributed lag non-linear models (DNLN), two-dimensional models developed to explore exposure-lag-response relationships along both the dimensions of exposure and lag (Gasparrini et al., 2010; Imai et al., 2015). These models use a cross-basis function, derived through a special tensor product of two independent functions, in order to analyze the exposure-response relationship and lag-response effect jointly. In our study, the effect of climatic parameters was modelled with a linear function, while the lag effect was modelled through a cubic basis spline with 4 degrees of freedom (df). The selection of the proper spline function for the lag-effect was based on the Akaike Information Criterion (AIC). We began the distributed lag models at lag 1 (the week before the week of diagnosis), hypothesizing that, since that WNV incubation period lasts 0–7 days (Rudolph et al., 2014), the risk should be null at lag 0 (week of diagnosis). The estimates can be plotted using a three-dimensional graph to show the Incidence Rate Ratio (IRR) along both exposure and lag dimension. Since the effect of climatic parameters was modelled as linear we estimated, for each lag, the IRR for an increase of 1 °C for the weekly average of minimum, mean and maximum temperatures and an increase of 5 mm for the weekly total precipitation. The lag-specific IRR was derived by exponentiating the estimated regression coefficient, namely the variation in log-rate, for a unit increase of each climatic parameter for all specific lag (lag 1–8). In addition, we estimated the overall cumulative effect, that is the sum of each specific lag contribution over the whole lag period and can be interpreted as the overall risk. To control further for residual seasonal confounding, we included a cubic basis spline function with 5 df of the week number of the year, able to capture the seasonal pattern of the case distribution observed during the transmission period.

In addition, during summer holidays people are more likely to move out from their area of residence for leisure reasons. Thus, change of geographical location between the case and the control period would violate an assumption of the case-crossover design and possibly introduce bias. The potential impact of this source of bias was assessed in a sensitivity analysis in which we adjusted for holiday periods, defined as the two weeks around the 15th of August.

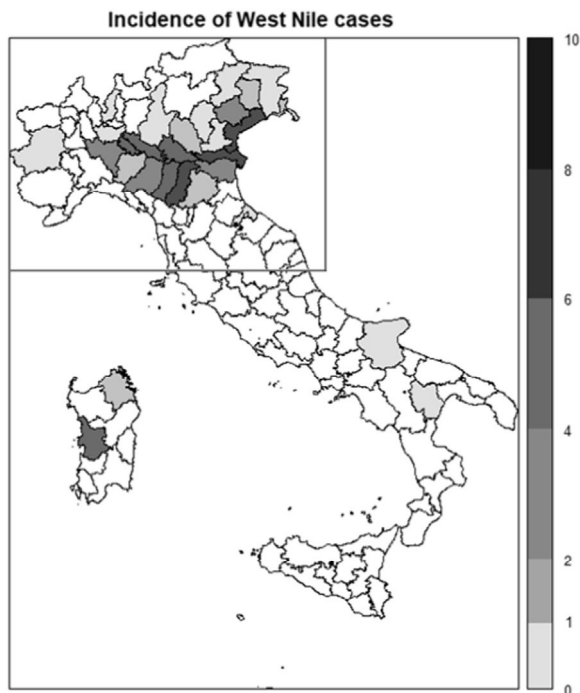


Fig. 1. Average of crude incidences of WNV infection per 1,000,000 person-years in Italian provinces during the study period. Framed area corresponds to the study area.

The software used to compute analysis is R, version 3.5.0 (R Development Core Team, 2018). The packages used for statistical analysis are “splines” “dlnm” and “gnm”.

### 3. Results

In total, 213 cases were diagnosed during the study period in Northern Italy and included in the case-crossover analysis. During 2010–2015 period, 25 provinces of Northern Italy out of 42 (60%) reported human cases of WNV infection. Fig. 1 shows the average of crude incidences of WNV infection per 1,000,000 inhabitants in each province over the 6-year period. Distribution of cases by week of the year (Fig. 2) shows that the WNV infection has a seasonal pattern in Italy, with all cases being notified during the summer/autumn period. All human cases occurred between the 28th and 44th week of the year with a peak at the end of August (36th week). This pattern has suggested the inclusion of the spline function of time to further adjust seasonal

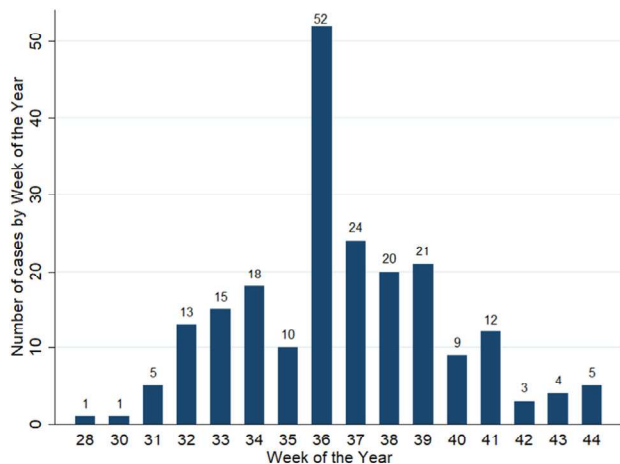


Fig. 2. Total number of WNV infection cases observed in Northern Italy during the study period (2010–2015) by week of the year (left) and by week and year (right).

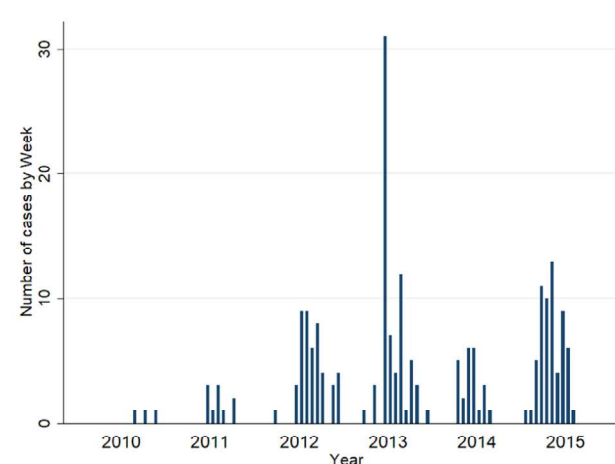
confounding.

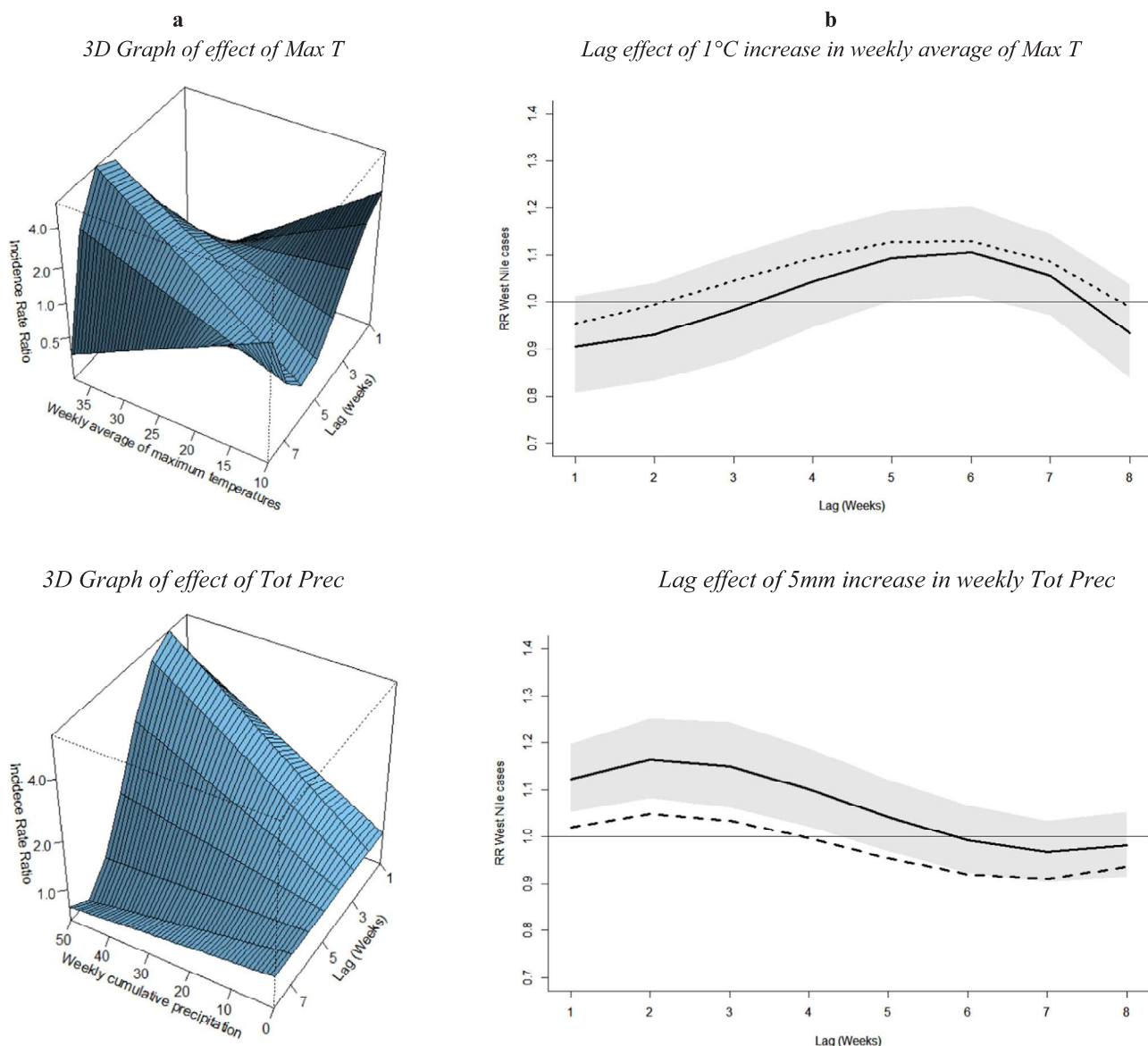
Results, both crude and adjusted for seasonality, conducted on climatic parameters recorded up to 8 weeks prior to the diagnosis in relation to the risk of WNV infection are shown in Fig. 3 and Table 1. The three-dimensional plots, show the entire surface of the adjusted IRRs in relation to maximum temperatures/precipitation at all lags considered (Fig. 3a). Fig. 3b shows the estimated effect of a unit increase in maximum temperatures and precipitation over the 8-week lag (continuous line: adjusted IRR, dashed line: crude IRR). Crude and adjusted lag-specific estimates for a unit increase in temperatures/precipitation are reported in Table 1. We found that the weekly average of maximum temperatures might affect the risk of WNV infection after 5 and 6 weeks (Fig. 3). As shown in Table 1, the highest effect on WNV incidence was observed considering maximum temperatures recorded in the 6th week prior to diagnosis (adjusted IRR for 1 °C increase in maximum temperatures at lag 6: 1.11; 95% CI 1.01–1.20). However, we did not find evidence of a positive overall cumulative effect for 1 °C increase in maximum temperatures on WNV infection risk in the following weeks (Table 1). Weekly average of mean and minimum temperatures was not associated with the risk of WNV infection at any lag (Table 1). Weekly total precipitation recorded at lag 1–4 resulted positively associated with the risk of WNV infection (Fig. 2b). As reported in Table 1, the maximum effect of precipitation was found with the precipitation recorded two weeks before diagnosis (lag 2) (adjusted IRR for 5 mm increase of weekly total precipitation at lag 2: 1.16; 95% CI 1.08–1.25). We found that 5 mm increase in weekly total precipitation was associated with a positive overall cumulative effect in the following 8 weeks: adjusted overall risk of 1.62 (95% CI 1.03–2.56). Lastly, when we adjusted for summer holidays in sensitivity analyses results were not affected more than marginally (results not shown).

### 4. Discussion

Our study revealed that cases in Northern Italy are notified between July and October, with a peak at the end of August. The transmission season is similar to the activity period (May–November) of mosquito *Culex pipiens*, the main WNV vector in Italy (Bisanzio et al., 2011).

Our study is, to our knowledge, the first to assess the lag-effect of meteorological exposures and risk of WNV infection in Italy, including all incident cases diagnosed in Northern Italy between 2010 and 2015. Methodologically, the main strength of this study is the application of DLNMs in the context of a time stratified case-crossover design in order to explore delayed effects of exposures. We further included in the model a seasonal term (namely a spline function of time) to enhance the study validity, as it has been shown that in presence of a strong seasonal pattern of exposures and outcomes, time-stratified case-crossover





**Fig. 3.** (a) (left) IRR2 (adjusted for seasonality) of WNV infection by weekly average of maximum temperatures (°C) and weekly total precipitation (mm), using a natural cubic spline–linear effect DLNM with 4 df basis cubic spline for lag and linear effect for exposure. (b) (right) The estimated IRR2 (adjusted for seasonality) and 95% confidence intervals in unit increase of weekly average of maximum/minimum temperature (1 °C) and of weekly total precipitation (5 mm) over 8 weeks of lag. Dashed line: IRR1 (not adjusted for seasonality).

studies might still be biased by residual seasonal confounding (Whitaker et al., 2007). Since we were interested in evaluating the short-term effect of the weekly variation of climatic parameters on the incidence of WNV infection from here onwards we will discuss only results adjusted for seasonality.

We found evidence of association, despite no overall cumulative effect, between maximum temperatures recorded in the 5th and 6th weeks prior to diagnosis (lags 5 and 6) and the incidence of WNV infection. Several studies have evaluated the effect of the temperatures on WNV ecology and transmission among mosquitoes, birds and humans in different areas worldwide (Gubler, 2007; Paz, 2015; Paz and Semenza, 2013), and many of them showed that temperatures may play an important role in the virus transmission cycle. However, only few studies have assessed the risk of WNV infection in humans in relation to temperatures with the specific aim of evaluating the lag effect. One correlation study conducted in Israel, Greece, Romania and Russia analyzed human cases of WNV infection notified during the summer of 2010 in relation to temperature anomalies, namely temperatures

recorded in 2010 compared with the perennial weekly average of 1981–2010. This study found an association between WNV cases and temperature at lag 0–1 (weeks) in Israel and Greece and at lag 3–4 (weeks) in Romania and Russia (Paz et al., 2013). One US study, a bi-directional case-crossover, not adjusted for seasonality, analyzed all incident cases of WNV infection notified between 2001 and 2005 (n = 16,298) in relation to the temperatures recorded in the 4 previous weeks, finding associations of similar strength for each lag (0–4 weeks) (Nile et al., 2009).

The lag of 5–6 weeks observed in our study might be explained by the complexity of the host/pathogen ecology. However, our study was not designed to assess the underlying mechanisms through which temperatures and precipitation may affect WNV infection, thus we can only speculate on the effects of climate parameters on vector and virus ecology.

It has been observed that the air temperature can augment virus replication rate and lead to higher viremia level in mosquito population (Reisen et al., 2006). Higher temperatures have been also shown to



**Table 1**  
Risk of WNV infection in relation to unit increase<sup>a</sup> in temperature and precipitation.

Lag (Weeks)	IRR1 <sup>b</sup>	95% CI	IRR2	95% CI
<b>1 °C increase in weekly average of maximum temperature</b>				
1	0.95	0.88–1.03	0.91	0.81–1.01
2	1.00	0.95–1.03	0.93	0.83–1.04
3	1.04	1.00–1.09	0.98	0.88–1.10
4	1.09	1.05–1.14	1.04	0.95–1.15
5	1.13	1.08–1.17	1.09	1.00–1.19
6	1.13	1.08–1.18	1.11	1.01–1.20
7	1.09	1.04–1.14	1.06	0.98–1.15
8	0.99	0.91–1.08	0.94	0.84–1.04
Cumulative effect	1.48	1.22–1.80	1.03	0.56–1.87
<b>1 °C increase in weekly average of mean temperature</b>				
1	0.95	0.86–1.05	0.88	0.77–1.01
2	1.00	0.96–1.04	0.90	0.79–1.03
3	1.05	1.00–1.11	0.95	0.83–1.09
4	1.10	1.05–1.15	1.02	0.90–1.15
5	1.13	1.08–1.18	1.08	0.97–1.20
6	1.13	1.08–1.19	1.09	0.99–1.21
7	1.09	1.03–1.15	1.04	0.94–1.15
8	1.00	0.91–1.12	0.91	0.79–1.04
Cumulative effect	1.53	1.23–1.92	0.86	0.41–1.80
<b>1 °C increase in weekly average of minimum temperature</b>				
1	0.96	0.86–1.07	0.91	0.80–1.05
2	1.01	0.96–1.06	0.90	0.79–1.03
3	1.06	1.00–1.12	0.93	0.81–1.07
4	1.10	1.05–1.15	0.98	0.86–1.12
5	1.12	1.08–1.17	1.03	0.92–1.15
6	1.12	1.07–1.18	1.04	0.93–1.17
7	1.09	1.03–1.16	1.00	0.89–1.12
8	1.02	0.92–1.15	0.88	0.75–1.02
Cumulative effect	1.60	1.24–2.07	0.71	0.32–1.56
<b>5 mm increase in weekly total precipitation</b>				
1	1.02	0.97–1.08	1.12	1.06–1.20
2	1.05	1.00–1.10	1.16	1.08–1.25
3	1.03	0.98–1.09	1.15	1.06–1.24
4	1.00	0.95–1.05	1.10	1.02–1.19
5	0.95	0.90–1.01	1.04	0.97–1.12
6	0.92	0.87–0.97	0.99	0.92–1.07
7	0.91	0.86–0.96	0.97	0.90–1.03
8	0.94	0.88–0.99	0.98	0.92–1.05
Cumulative effect	0.82	0.57–1.14	1.62	1.03–2.56

<sup>a</sup> Estimates for a unit increase are derived by exponentiating the estimated regression coefficient, namely the variation in log-rate, for a unit increase of meteorological variables. Estimates for  $n$ -fold unit increase is obtainable by raising the estimate to the  $n$ -power.

<sup>b</sup> IRR1: Crude Incidence Rate Ratio; IRR2: Incidence Rate Ratio adjusted for seasonality; CI: Confidence Interval.

impact the vector transmission rate, by shortening the extrinsic incubation period (namely “the time from ingestion of an infectious bloodmeal until a mosquito is capable of transmitting virus infection to a susceptible organism”) (Reisen, 1989; Reisen et al., 2006). In addition, elevated temperatures can cause an expansion of the absolute number of mosquitoes and affect their feeding behaviours (Bisanzio et al., 2011; Conte et al., 2015). Thus, higher temperatures are believed to first impact the virus transmission in the enzootic cycle among mosquitoes and birds (Kilpatrick et al., 2008; Reisen et al., 2006) and, second, to affect the expansion of the proportion of infective mosquitoes, on which depend the human infection. The aforementioned pathways intrinsically imply a latency of the effect that, in addition to an incubation period of 0–7 days of human infection (Rudolph et al., 2014), might explain the overall latency of 5–6 weeks observed between increased temperatures and higher incidence of WNV infection cases.

However, it is noteworthy that the whole lag pattern presents negative point estimates at lag 1–2 and that the overall cumulative effect estimate is close to zero. For these reasons we cannot exclude that our

findings of association between increased maximum temperatures and incidence of WNV infection at lag 5–6 might be due to chance.

Our results revealed an association between WNV infection and total precipitation recorded between the 1 and 4 weeks prior the diagnosis (lag 1–4). Levels of precipitations are believed to affect the patterns and the transmission of WNV (Paz, 2015). However, findings about the relationship between precipitation and incidence of WNV cases are contradictory. Some studies reported that above-average precipitation can lead to higher risk of WNV outbreaks by expanding mosquitoes (Di Sabatino et al., 2014; Nile et al., 2009). On the contrary, other studies found that drought periods can induce outbreaks favoring the bird-to-bird viral transmission by facilitating the concentration of avian species in the few existing pools (Shaman et al., 2005). It is plausible that the response to precipitation might change over different geographical areas, depending on the differences in the characteristics of the local environment and in the ecology of vectors (Shaman et al., 2002; Paz, 2015). Our results of associations between WNV infection cases and increased precipitation at lag 1–4 (weeks) can be due to the close relationship between aquatic environment and mosquito proliferation. Intermediate stages of *Culex* mosquitoes, such as larvae, are water dependent, and therefore, precipitation might be important, especially in drought periods such as summer, to create and maintain water pools that are necessary for the development of mosquitoes. Accordingly, an observational study reported that the WNV outbreak recorded in 2010 in central Macedonia, Greece, was preceded by unusually precipitation (Danis et al., 2011).

Our study has three main limitations. First, we had information on the week but not on the day of diagnosis. Thus, we could not date back the exposure history starting from the day of symptoms onset, but only from the week preceding the week of the diagnosis. However, our study aligns with most of environmental studies conducted on infectious diseases, as typically surveillance systems for communicable diseases notify cases on a weekly scale. Second, since we had no information about the municipality but only about the province of residence of the cases, we linked each case to the meteorological station of the capital of its province in order to obtain data on the corresponding environmental exposures. This linkage might have introduced some non-negligible degree of exposure misclassification. However, since in case-crossover analysis the same subject is used both as case and as its own control, misclassification is likely to be non-directional, which would likely lead to conservative estimates. Third, the reason of the diagnosis (asymptomatic subjects: WNV positive blood; symptomatic subjects: West Nile Fever or West Nile Neuroinvasive Disease) was not available at the individual level. Asymptomatic subjects, such as blood donors, can be diagnosed during the incubation period, and therefore the lag-effect of environmental exposures might be different between asymptomatic and symptomatic groups. However, WNV infection cases diagnosed among the blood donors represent a minority of cases identified through the surveillance system. For instance, only 13 out of 61 cases (21% of the total) observed in Italy in 2015 were blood donors (ISS, 2015).

## 5. Conclusions

In conclusion, our results suggest that high temperatures might be associated with the incidence of WNV infection after a lag of 5–6 weeks, while heavy precipitation after a lag of 2–3 weeks. These results strengthen the evidence that the WNV is a climate-sensitive disease in an area where the process of endemization has recently started and underline that climatic parameters might be useful for detecting areas and periods of the year potentially characterized by a higher incidence of WNV infection.

## Acknowledgments

Dr Gasparrini was supported by the Medical Research Council (Grant ID: MR/M022625/1). Data from The European Surveillance

System – TESSy, provided by Italy and released by ECDC. The views and opinions of the authors expressed herein do not necessarily state or reflect those of ECDC. The accuracy of the authors' statistical analysis and the findings they report are not the responsibility of ECDC. ECDC is not responsible for conclusions or opinions drawn from the data provided. ECDC is not responsible for the correctness of the data and for data management, data merging and data collection after provision of the data. ECDC shall not be held liable for improper or incorrect use of the data.

### Competing interests statement

The authors have no competing financial interests to declare.

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# CHAPTER 4

## Enhanced West Nile Virus Circulation in the Emilia-Romagna and Lombardy Regions (Northern Italy) in 2018 Detected by Entomological Surveillance

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*Frontiers in Veterinary Science*, 2020 May; **7**:243

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**M. Calzolari:** Design of the surveillance system, Conceptualization, Data Curation, Statistical Analysis, Writing – original draft

**P. Angelini, R. Cagarelli, D. Cereda, M.P. Cerioli, M. Chiari, G. Galletti, M. Tamba, R. Bellini, and D. Lelli:** Design of the surveillance system, Writing – review & editing

**S. Canziani, and T. Trogu:** Data Curation, Writing – review & editing

**P. Bonilauri and D. Torri:** Molecular Analysis, Writing – review & editing

**L. Bolzoni, and A. Albieri:** Data Curation, Statistical Analysis, Writing – review & editing



# Enhanced West Nile Virus Circulation in the Emilia-Romagna and Lombardy Regions (Northern Italy) in 2018 Detected by Entomological Surveillance

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### Specialty section:

This article was submitted to  
Veterinary Infectious Diseases,  
a section of the journal  
Frontiers in Veterinary Science

Received: 25 October 2019

Accepted: 09 April 2020

Published: 05 May 2020

### Citation:

Calzolari M, Angelini P, Bolzoni L,  
Bonilauri P, Cagarelli R, Canziani S,  
Cereda D, Cerioli MP, Chiari M,  
Galletti G, Moirano G, Tamba M,  
Torri D, Trogu T, Albieri A, Bellini R and  
Lelli D (2020) Enhanced West Nile  
Virus Circulation in the  
Emilia-Romagna and Lombardy  
Regions (Northern Italy) in 2018  
Detected by Entomological  
Surveillance. *Front. Vet. Sci.* 7:243.  
doi: 10.3389/fvets.2020.00243

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With several human cases reported annually since 2008 and the unapparent risk of infection of blood donors, the West Nile virus (WNV) is emerging as an important health issue in Europe. Italy, as well as other European countries, experienced a recrudescence of the virus circulation in 2018, which led to an increased number of human cases. An integrated surveillance plan was activated in the Emilia-Romagna and Lombardy regions (Northern Italy) since 2008 in order to monitor the intensity and timing of WNV circulation. A fundamental part of this plan consists in entomological surveillance. In 2018, the surveillance plan made it possible to collect 385,293 mosquitoes in 163 stations in the two Regions. In total 269,147 *Culex* mosquitoes were grouped into 2,337 pools and tested for WNV, which was detected in 232 pools. Circulation started in the central part of the Emilia-Romagna region in the middle of June, about one month before the previous seasons. Circulation suddenly expanded to the rest of the region and reached the Lombardy region in the middle of July. WNV circulated more intensively in the eastern part of the surveyed area, as confirmed by the highest number of human cases. A relationship between the number of mosquitoes collected and the virus incidence emerged, but the data obtained highlighted that the probability of detecting the virus in a given site was less than expected with a higher number of collected mosquitoes. A significant relationship was observed between the temperature recorded one week before the sampling and the number of collected mosquitoes, as well as between the estimated number of WNV-positive mosquitoes and the temperature recorded two weeks before the sampling. The two weeks delay in the influence of temperature on the positive mosquitoes is in line with the time of the virus extrinsic incubation in the mosquito. This finding confirms that

temperature is one of the principal drivers in WNV mosquito infection. The surveillance system demonstrated the ability to detect the virus circulation early, particularly in areas where circulation was more intense. This allowed evaluating the effect of mosquito abundance and weather factors on virus circulation.

**Keywords:** West Nile virus, *Culex pipiens*, surveillance, infection rate, temperature, one-health

## INTRODUCTION

The West Nile Virus (WNV) is a flavivirus that circulates in the environment among birds and mosquitoes, but can infect other animals such as equids and humans as dead-end hosts. In humans, virus infection is generally, asymptomatic, but about 20% of infected subjects can develop a febrile illness, which can evolve into a neuro-invasive disease (WNND) in a minority of cases (less than 1% of infected subjects). In addition to symptomatic human cases, the presence of infected, but asymptomatic, blood and organ donors is a primary health issue (1).

After sporadic reports in the '90s in Romania (2) and in Italy (3), as from 2008, the circulation of the virus was recorded at a different extent every season in Europe. In 2018, an alarming recrudescence of the virus circulation was recorded in Europe (4). This recrudescence was also noticed in Northern Italy, including the Emilia-Romagna and Lombardy regions, where an interdisciplinary surveillance plan targeting WNV was set up in 2008 and implemented over the years (5–10).

The plan focuses on the early detection and the pinpointing of the virus circulation area, including those areas where the virus did not circulate the previous season. Over time, the system has shown that it is capable of WNV circulation's early detection at provincial scale (6) and it is economically sustainable (11). Moreover, the plan was able to detect other arboviruses circulating in the surveyed area (12).

The plan has a one-health approach in order to maximize early detection, involving searching for the virus in humans, horses, birds, and mosquitoes. Entomological surveillance is a fundamental part of the plan, characterized by regular scheduling of samplings, and the precise geographic characterization of obtained samples. This permits a fine characterization of the virus circulation on the surveyed area. In this work, we characterize the extraordinary WNV circulation observed in 2018, utilizing data from entomological surveillance obtained in the Emilia-Romagna and Lombardy regions. In particular, we describe the relationship between WNV detection and the abundance of collected mosquitoes, as well as the influence of weather conditions, such as weekly maximum temperatures and number of wet days, on the virus circulation in mosquitoes.

## MATERIALS AND METHODS

### Surveyed Area

The surveyed area included the plan area of the Emilia-Romagna and Lombardy regions. This is a large portion of Italy's main plain, the Pianura Padana (or Pianura Padano-Veneta), which includes about 24,000 km<sup>2</sup> (51.3%) of the total area of 44,700

km<sup>2</sup>. This area encompasses about 10 million inhabitants, with many urban areas, including two of the biggest Italian cities (Milan and Bologna), as well as several important industrial districts. This area has a relevant livestock patrimony and is one of the more important agricultural areas in Italy; distinctive cultivations such as rice fields, vineyards and orchards are highly represented in specific areas.

In general, a strong anthropic modification, with the abundant presence of industrial and urban settlements, characterizes the surveyed area. The rural part of the territory is connoted by an intensive agriculture and animal husbandry, with few hedges, rare scattered trees, and a dense irrigation network. Natural areas are rare, mainly represented by river borders, characterized by riparian vegetation, or re-naturalized areas. The eastern part of the surveyed area ends in the Adriatic Sea, where some of the largest wetlands in Europe are present (Valli di Comacchio and the Po River Delta); pinewood and typical Mediterranean vegetation can be found in this zone.

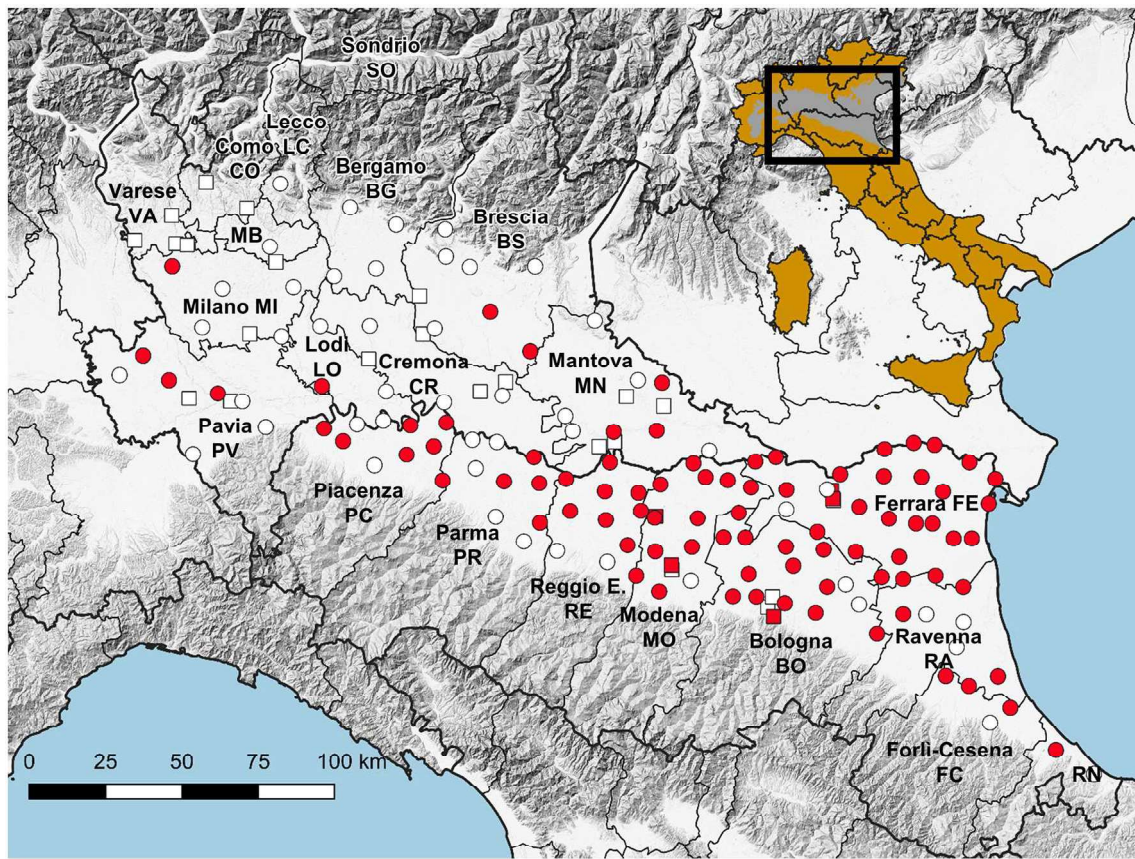
Trapping sites, with different densities, were selected to cover the entire plain area of the two regions: 95 traps in Emilia-Romagna (surveillance grid of 110 km<sup>2</sup>) and 39 traps in Lombardy (surveillance grid of 400 km<sup>2</sup>) (Table 1, Figure 1). These traps worked regularly throughout the surveillance season (seasonal traps). Surveillance was focused on rural areas, semi-natural, rural, or peri-urban locations in Emilia-Romagna, and farms in Lombardy. A supplementary effort was carried out by activating 23 specific traps along the surveillance season in Lombardy, and 9 supplementary traps, which worked once (on August 10th), among selected urban areas in Emilia-Romagna.

### Mosquito Analysis

We collected mosquitoes in fixed geo-referenced stations using attractive traps baited by carbon dioxide, working overnight, roughly from 5:00 p.m. to 9:00 a.m. Every site regularly included in the surveillance was sampled every fortnight. Mosquitoes

**TABLE 1** | Features of surveillance in the two surveyed regions.

	Emilia-Romagna	Lombardy
Number of seasonal traps	95	39
Number of extra traps	9	23
Maximum number of mosquitoes per pool	200	100
Maximum number of mosquitoes tested per sampling	all sampled	1000
Start date	June 12	June 4
End date	October 16	October 25



**FIGURE 1 |** Map showing the location of traps working throughout the season (circles) and for part of the season (squares), with reference to WNV detections (red), and the reference of the surveyed area on a map of Italy in which the Pianura Padano-Veneta is depicted in gray.

were identified using morphological characteristics according to the classification key of Severini et al. (13). Due to the used trap model, baited with carbon dioxide, we collected almost exclusively host seeking females, the catching of males was very rare. Identified mosquitoes were counted and pooled according to date, location, and species. The maximum number of specimens per pool was 200 in Emilia-Romagna and 100 in Lombardy. A maximum of 1,000 specimens per species was tested for every sample in Lombardy. Pooled mosquitoes belonging to the genus *Culex*, and selected pools of other species, were prepared and submitted to biomolecular tests as described elsewhere (14).

In brief, pooled mosquitoes were ground by a vortex mixer with 2-3 4.5-mm-diameter copper-plated round balls (H&N Sport, Münden, Germany) in 2 ml of PBS. After centrifugation, 200  $\mu$ L aliquots were collected and submitted to biomolecular analysis, for extracting and retrotranscribing RNA. We tested samples by a WNV real-time RT-PCR, according to the method described by Tang et al. (15). This was confirmed by the protocol of Del Amo et al. (16) and by an universal PCR protocol for the identification of flaviviruses (17).

We estimated the number of positive mosquitoes for every site and the day of sampling utilizing the Maximal Likelihood

Estimation (MLE) of the PooledInfRate 4.0 excel add-in (18), multiplying the MLE of the infectious rate for the number of collected mosquitoes, and rounding up the obtained result. MLE cannot be estimated when all sample pools test positive. In this case, we approximated the number of infected mosquitoes using the Minimum Infection Rate (MIR), assuming that only one positive mosquito was present in each pool.

We tested the relationship between the fraction of infected mosquitoes collected in each province, with at least two working seasonal traps, and the incidence of WNV cases in the same province through a linear model. Additionally, we checked the hypothesis of normality in the distribution of model residuals through the Shapiro-Wilk test.

### Mosquito Abundance and WNV Circulation

We tested the effect of mosquito abundance in a given area (using the catches per trap as a proxy of abundance) on the probability of WNV circulation in the same area in 2018. To guarantee comparable catching efforts, considering the start/end of trapping seasons differed among traps, we used the total number of mosquitoes caught in each trap in July and August (for a total of four sampling sessions) as a measure of mosquito abundance. Traps with less than four sampling sessions in the

July–August time window were excluded from the analyses. We built a generalized linear model (GLM) with binomial error distribution and logit link function to estimate the probability of observing a WNV-positive trap in 2018, using the observed number of mosquitoes caught in each trap and the region where the trap was deployed as explanatory variables. In this model, the effect on the GLM of caught mosquitoes (namely,  $M_{OBS}$ ) represents the marginal increase in the probability of observing a WNV-positive trap due to an additional mosquito caught (in the logit link).

To test the effect of mosquito abundance on WNV circulation, we built a null model where the probability of each mosquito to test WNV-positive only depends on the region where the mosquito was caught and not on the abundance of mosquitoes in the trap area. These probabilities, namely  $p_{NULL\_ER}$  and  $p_{NULL\_LO}$ , were estimated by maximizing the likelihood of obtaining the observed pattern of positive traps in 2018, given the number of mosquitoes caught in each trap in Emilia-Romagna and Lombardy, respectively. We then randomly generated 10,000 synthetic samples where we assigned a WNV-positive/negative status to each mosquito caught in Emilia-Romagna [resp. Lombardy] with probabilities  $p_{NULL\_ER}$  [resp.  $p_{NULL\_LO}$ ]. Analogously to the observed sample, we estimated for each synthetic sample the effect of the number of mosquitoes caught on the probability of observing a WNV-positive trap (namely,  $M_{NULL}$ ) through GLMs. Thus, we generated a distribution of the estimated  $M_{NULL}$  which represents the null hypothesis where mosquito abundance in a given area does not affect the probability of each single mosquito testing WNV-positive. We then tested the observed effect,  $M_{OBS}$ , against the distribution of  $M_{NULL}$ . If  $M_{OBS}$  is significantly higher [resp. smaller] than  $M_{NULL}$  (i.e., it falls outside the 95% range of the  $M_{NULL}$  distribution), then mosquito abundance provides an amplification [resp. dilution] effect on WNV circulation. Analyses were performed with R software.

In order to spatially characterize mosquito abundance, we interpolated the number of mosquitoes sampled in each seasonal trap between July–August (expressed as a natural logarithm) on a map, using the inverse distance weighting method (IDW). The virus circulation's spatial pattern was obtained using positive trap location with the kernel density estimation (KDE) method, with the bandwidth size of 15 km. The area of more intense WNV circulation was estimated through the 50% volume contour of the obtained WNV KDE surface. We used QGIS 3 software (<http://www.qgis.org>) to perform these analyses.

## Meteorological Factors, Mosquito Abundance and Infection Rate

We retrieved daily maximum temperatures and precipitations data from the ECA&D dataset (19) and extracted the data for every surveyed trap site in 2018. We aimed to estimate the association of meteorological parameters with the number of sampled *Culex* mosquitoes and with the infection rate (namely the proportion of WNV-positive mosquitoes). Since the effect of meteorological parameters on the outcomes of interest might be delayed (lag-effect), we focused on the meteorological parameters

**TABLE 2 |** Mosquitoes sampled during the 2018 surveillance season.

Species	Emilia-Romagna (%)	Lombardy (%)	Extra traps	Total (%)
<i>Ae. albopictus</i>	4557 (1.6)	1743 (1.7)	665 (19.3)	6965 (1.8)
<i>Ae. berlandi</i>	1 (<0.5)			1 (<0.5)
<i>Ae. caspius</i>	43526 (15.7)	45299 (43.4)	118 (3.4)	88943 (23.1)
<i>Ae. cinereus</i>	3 (<0.5)			3 (<0.5)
<i>Ae. geniculatus</i>	15 (<0.5)			15 (<0.5)
<i>Ae. vexans</i>	7634 (2.8)	606 (0.6)	412 (11.9)	8652 (2.2)
<i>An. maculipennis s.l.</i>	753 (<0.5)	7007 (6.7)	1 (<0.5)	7761 (2.0)
<i>An. plumbeus</i>	10 (<0.5)	4 (<0.5)		14 (<0.5)
<i>Cq. richiardii</i>	658 (0.2)			658 (0.2)
<i>Cs. annulate</i>	3 (<0.5)	32 (<0.5)		35 (<0.5)
<i>Cs. longiareolata</i>	1 (<0.5)			1 (<0.5)
<i>Cx. modestus</i>	35 (<0.5)			35 (<0.5)
<i>Cx. pipiens</i>	220255 (79.4)	49697 (47.6)	2258 (65.4)	272210 (70.7)
Total	277451	104338	3454	385293

recorded up to 4 weeks (lag 1–4) before the night of sampling. Starting from the day before the sampling, we computed the weekly average of maximum temperatures and the weekly number of wet days (number of days in a week with precipitation > 0.5 mm).

Analyses were performed in the framework of the generalized linear mixed models (GLMMs) (20). Specifically, we applied a GLMM including a site-specific and week-specific random effect to account for site and temporal heterogeneity. The abundance of mosquitoes sampled per trap and night was analyzed by applying a linear mixed model with a normal distribution and identity link function. We used the natural logarithm of the count of sampled mosquitoes as the dependent variable and the meteorological parameters recorded up to 4 weeks before the sampling as predictors (lag 1–4). The per trap and night infection rate was analyzed by applying a generalized linear mixed model with Poisson distribution and log link function. We used the estimate of infected mosquitoes as the dependent variable, the number of sampled mosquitoes as offset and the meteorological parameters recorded up to 4 weeks before the sampling as predictors (lag 1–4). The association between temperatures and outcomes was assessed by estimating the coefficient for a unit increase (1°C) in the weekly average of maximum temperatures. The association between precipitations and outcomes was assessed by estimating the coefficient for a unit increase (1 day) in the weekly number of wet days. Analyses were performed with R software (*lme4* package).

## RESULTS

### Mosquito Results

385,293 mosquitoes belonging to 13 species were sampled between June 4 and October 25, (Table 2). Mosquitoes of the *Culex* genus were grouped into 2,337 pools, of which 232

**TABLE 3 |** Tested and WNV-positive mosquito pools for the 2018 surveillance season.

Species	N	E-R		Lom			Extra-traps			Total		
		Pools	pos	N	pools	pos	N	pools	pos	N	pools	pos
<i>Ae. albopictus</i>				16	2		177	11		193	13	
<i>Ae. caspius</i>	8211	160		5	1		107	5		8323	166	
<i>Cx. modestus</i>	35	6								35	6	
<i>Cx. pipiens</i>	220255	1554	194	46599	700	32	2258	77	6	269112	2331	232
Total	228501	1720	194	46620	703	32	2542	93	6	277663	2516	232

**TABLE 4 |** Details of entomological surveillance at provincial level during the 2018 surveillance season.

Region	Prov.	First WNV/+ pool	Last WNV/+ pool	Days	Average sampled*	CI	Total sampled	Est. positive mosq.	WNND cases
Emilia-Romagna	BO	19/6	7/9	80	227	180-273	30603	49	41
	FC	5/7	2/8	28	103	38-168	2780	2	2
	FE	19/6	6/9	79	248	201-295	61730	89	14
	MO	3/7	14/8	42	243	182-303	25864	39	23
	PC	10/7	2/10	84	380	292-468	37619	8	2
	PR	17/7	28/8	42	303	202-404	24257	14	1
	RA	24/7	21/8	28	124	88-160	6574	5	13
	RE	15/6	24/8	70	334	241-428	30067	25	5
	RN	31/7	31/7		88	22-154	1584	1	0
Lombardy	BS	26/7	23/8	28	185	115-255	13811	3	1
	LO	2/8	2/8	0	152	70-234	4103	1	1
	MI	7/8	6/9	30	62	38-85	2895	2	6
	MN	17/7	20/8	34	164	98-230	14047	14	4
	PV	30/7	20/8	21	175	120-230	11919	17	1
	BG				40	16-63	1472	0	0
	CO				–		20	0	1
	CR				92	38-147	2444	0	2
	LC				57	12-102	341	0	0
	MB				6	2-10	48	0	0
	VA				–		32	0	0

\* In traps which work throughout the season.

tested positive for lineage 2 of the WNV. The vast majority of tested pools and all pools which tested positive were composed of *Culex (Cx.) pipiens* (Table 3). The 134 seasonal traps collected 269,952 *Cx. pipiens* specimens; of these, 266,854 were tested in 2,254 pools, and 226 were WNV-positive. From among the 134 seasonal traps, 85 collected at least one WNV-positive pool (Figure 1). A total of 1,101 mosquitoes were sampled in the 9 extra traps activated in cities (Bologna, Modena, and Ferrara) in August. All mosquitoes from these samples (823 *Cx. pipiens*, 171 *Aedes (Ae.) albopictus*, and 107 *Ae. caspius*) were tested, and 6 out of the 14 *Cx. pipiens* pools resulted positive. No positive pools were detected in the other extra traps only activated for part of the season, mainly in northern Lombardy. In order to evaluate the possible involvement of *Ae. caspius* in virus circulation, we also tested 160 selected pools of this species (for a total of 8,211 specimens) collected between July 17 and August 14. All gave negative results.

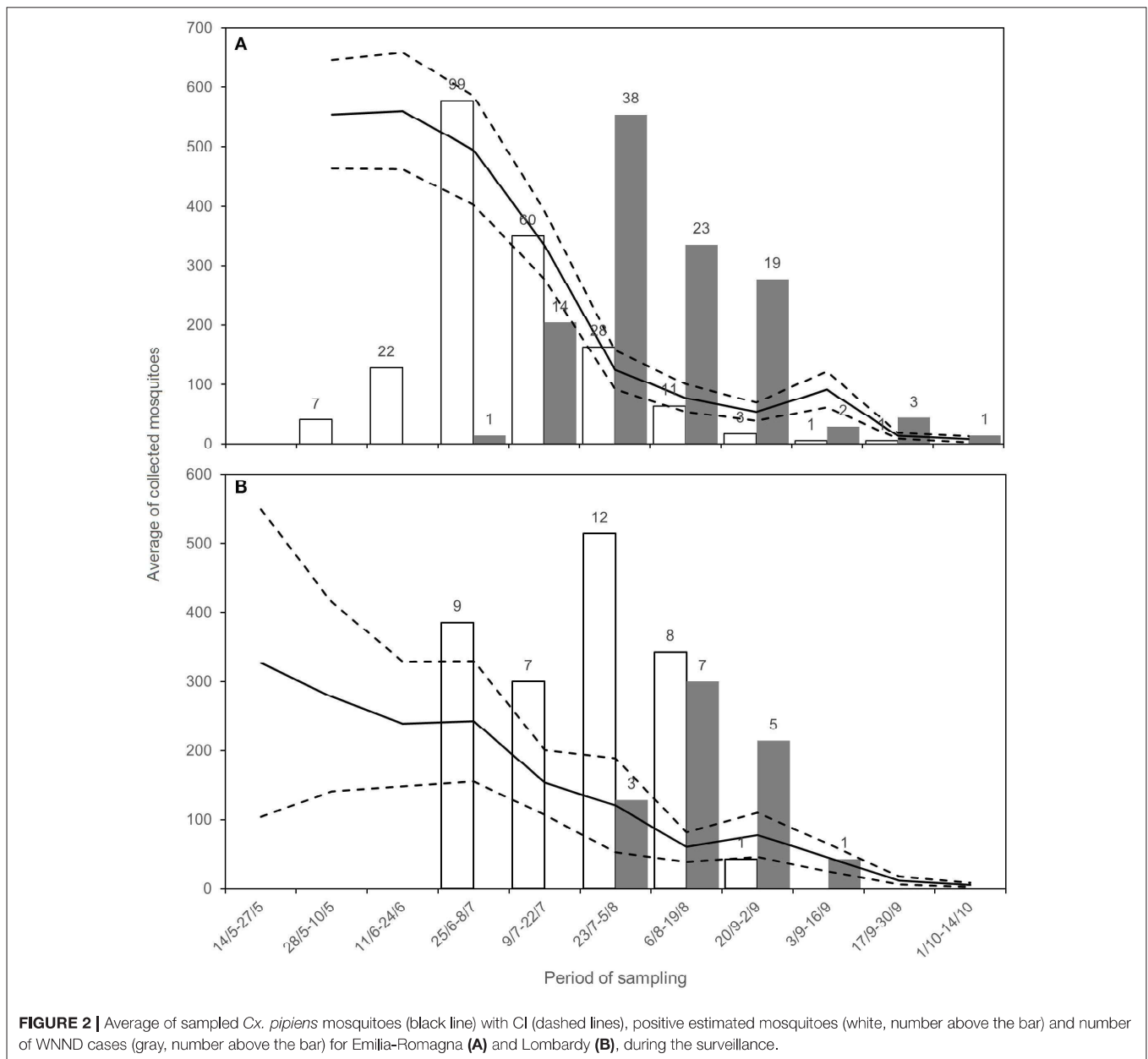
The average of collected mosquitoes per sample at provincial level showed the highest values in the western part of Emilia-Romagna, in Piacenza, Reggio Emilia, and Parma provinces (Table 4). A significant difference in the number of collected mosquitoes was recorded between Lombardy and Emilia-Romagna traps (considering only seasonal traps:  $\mu_{E-R} = 258$ , CI 233-283,  $\mu_{LOM} = 135$ , CI 110-159,  $t = 5.9016$ ,  $p = 4.6e-9$ ).

## Characterization of Virus Circulation

In 2018, WNV circulation was first detected on June 15 in Emilia-Romagna and on July 17 in Lombardy. We collected the last positive pools on September 6 and October 2 in Lombardy and Emilia-Romagna, respectively (Table 4). The epidemic curve of WNND human cases was postponed with respect to the detection of positive mosquitoes (Figure 2).

Only taking seasonal trap data into account, we evaluated the intensity of circulation at the provincial level considering the number of estimated WNV positive mosquitoes on total





**FIGURE 2 |** Average of sampled *Cx. pipiens* mosquitoes (black line) with CI (dashed lines), positive estimated mosquitoes (white, number above the bar) and number of WNV cases (gray, number above the bar) for Emilia-Romagna (A) and Lombardy (B), during the surveillance.

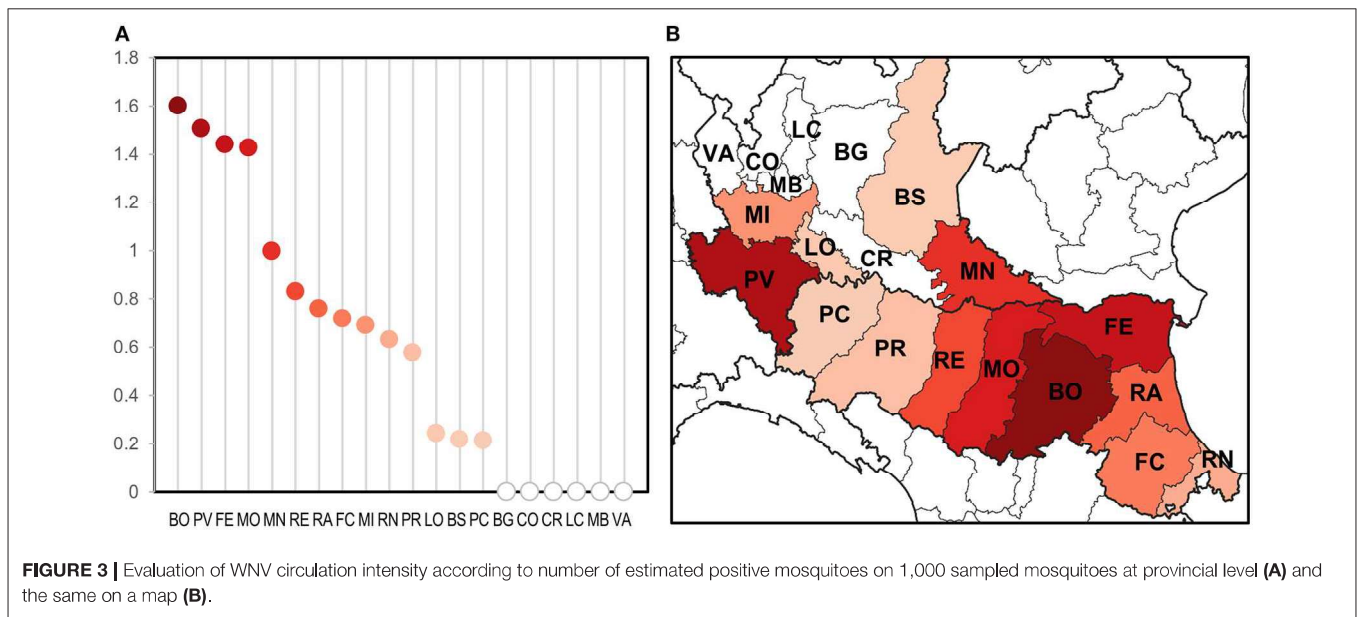
mosquitoes tested (Figure 3). This highlighted groups of provinces with a different circulation intensity: (i) a group of provinces with a more intense circulation (including central Emilia-Romagna and Pavia); (ii) a group of provinces with an intermediate circulation intensity (in the central and eastern surveillance zones); (iii) a group with a low circulation intensity (Lodi, Brescia, and Piacenza); and (iv) a group in which the virus was undetected (northern Lombardy). Provinces with a more intense circulation often recorded an early and more prolonged virus circulation (Table 4), and a major number of WNV cases.

We found the existence of a significant relationship between the rate of infected mosquitoes collected in each province and the incidence of WNV cases in the same province ( $p = 0.0013$ ). Specifically, we estimated that an increase of 1% in the rate of

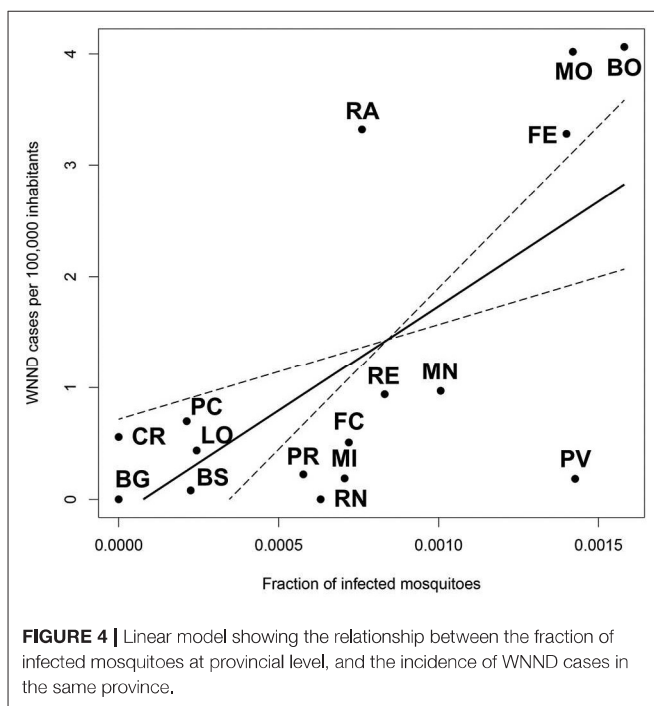
WNV positive pools leads to an additional 1.88 WNV cases per 100,000 inhabitants (Figure 4).

### Relationship Between the Number of Collected Mosquitoes and WNV Incidence

We found a significantly higher probability of finding WNV-positive traps in Emilia-Romagna with respect to Lombardy ( $p$ -value =  $8.7e-5$ ). We also estimated the marginal increase in the probability of observing a WNV-positive trap due to an additional mosquito caught as  $M_{OBS} = 0.00102$  ( $p$ -value = 0.0021). Figure 5B shows the distribution of the marginal increase in the probability of observing a WNV-positive trap due to an additional mosquito caught obtained with the null model ( $M_{NULL}$ , gray bars) compared to  $M_{OBS}$  (black line). We



**FIGURE 3 |** Evaluation of WNV circulation intensity according to number of estimated positive mosquitoes on 1,000 sampled mosquitoes at provincial level (A) and the same on a map (B).



**FIGURE 4 |** Linear model showing the relationship between the fraction of infected mosquitoes at provincial level, and the incidence of WNND cases in the same province.

found that  $M_{OBS}$  is significantly lower than  $M_{NULL}$  ( $p$ -value = 0.0232), this means that real number of captured mosquitoes affects less than in the simulations the probability to detect WNV in a trap, suggesting that mosquito abundance in a given site provides a dilution effect on WNV circulation. **Figure 5** displays the probabilities of observing a WNV-positive trap as a function of the number of mosquitoes per trap in Lombardy (panel A) and Emilia-Romagna (panel C), estimated through the observed data (black solid lines: best fit, dashed lines: 95% confidence interval) and the null model (white lines: median, gray areas: 95%

interval). Panels A and C in **Figure 5** show that the probability of finding WNV-positive traps is higher than expected when the abundance of caught mosquitoes is low (i.e., black lines are higher than the white lines), while the opposite is true when the abundance of caught mosquitoes is high (i.e., black lines are lower than the white lines).

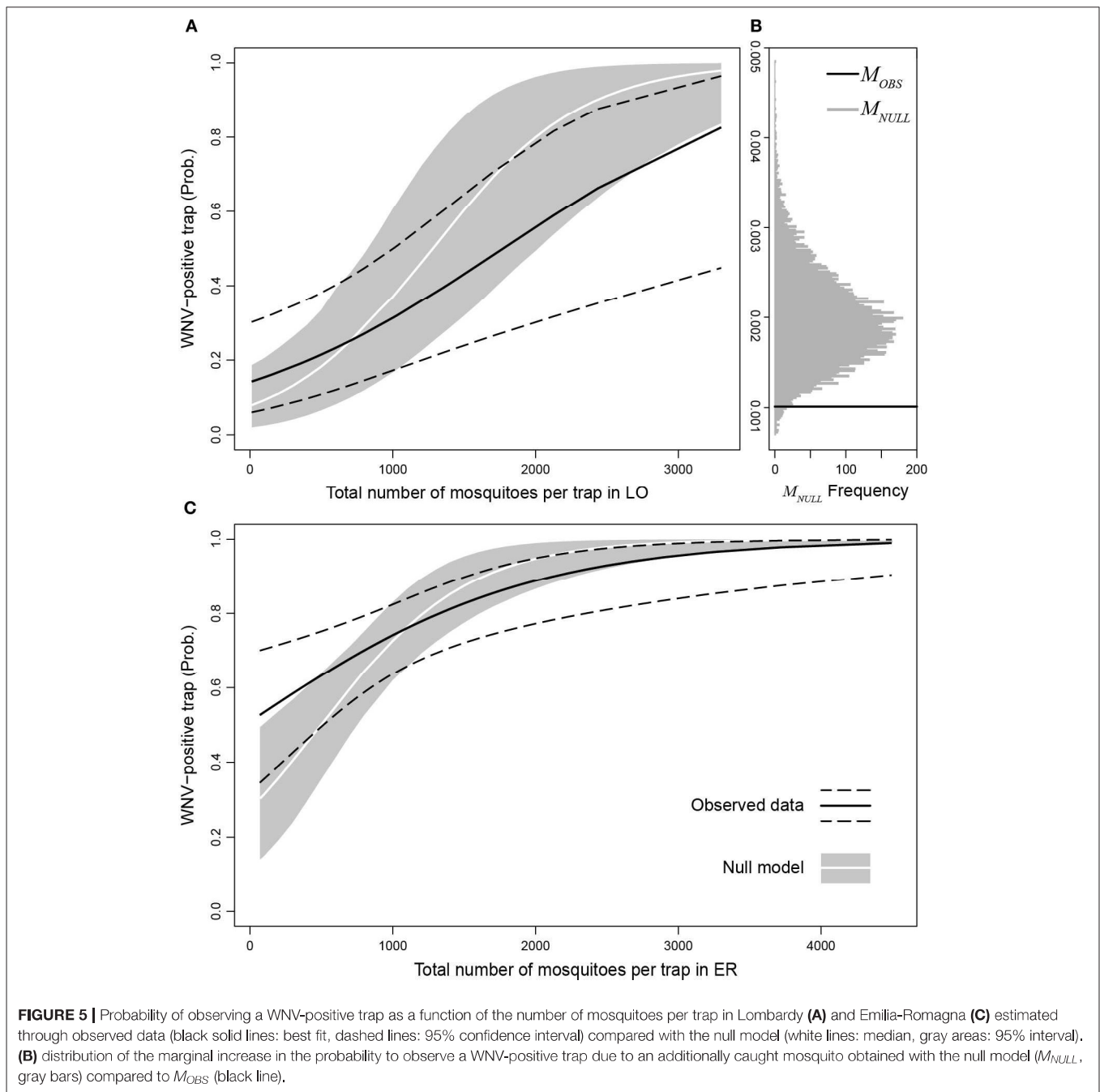
Despite the probability of finding that WNV increases with the number of mosquitoes (i.e.,  $M_{OBS} > 0$ ), the probability of a single mosquito being infected decreases as the number of collected mosquitoes increases. Thus, the estimated probability of a single mosquito collected in one site being infected decreased with the increase in the number of collected mosquitoes in the same site.

Fifty percent of the positive traps' KDE revealed a single hot spot in Emilia-Romagna where WNV circulation is more intense (in orange in **Figure 6**). Overlaying this map onto the map of sampled mosquitoes, directly related to mosquito abundance, highlighted that areas where mosquitoes are most abundant are not necessarily included in this WNV hot spot (**Figure 6**).

### Association Between Weather Data and WNV Circulation

**Table 5** and **Figure 7** report the  $\beta$  coefficients and 95% CI for the fixed effects of lagged meteorological parameters. Fixed effect coefficients are average estimates from among all sampled traps of the association between a unit increase in the meteorological parameters and the outcomes.  $\beta$  coefficients are estimated under the null hypothesis of absence of association ( $\beta = 0$ ), thus a positive estimate of  $\beta$  coefficient suggests that an increase in the meteorological parameter is associated with an increase in the outcome under study (number of sampled mosquitoes or infection rate).

As shown in **Table 5** and **Figure 7**, we found an association between the logarithm of the number of sampled mosquitoes and the weekly average of maximum temperatures recorded the week

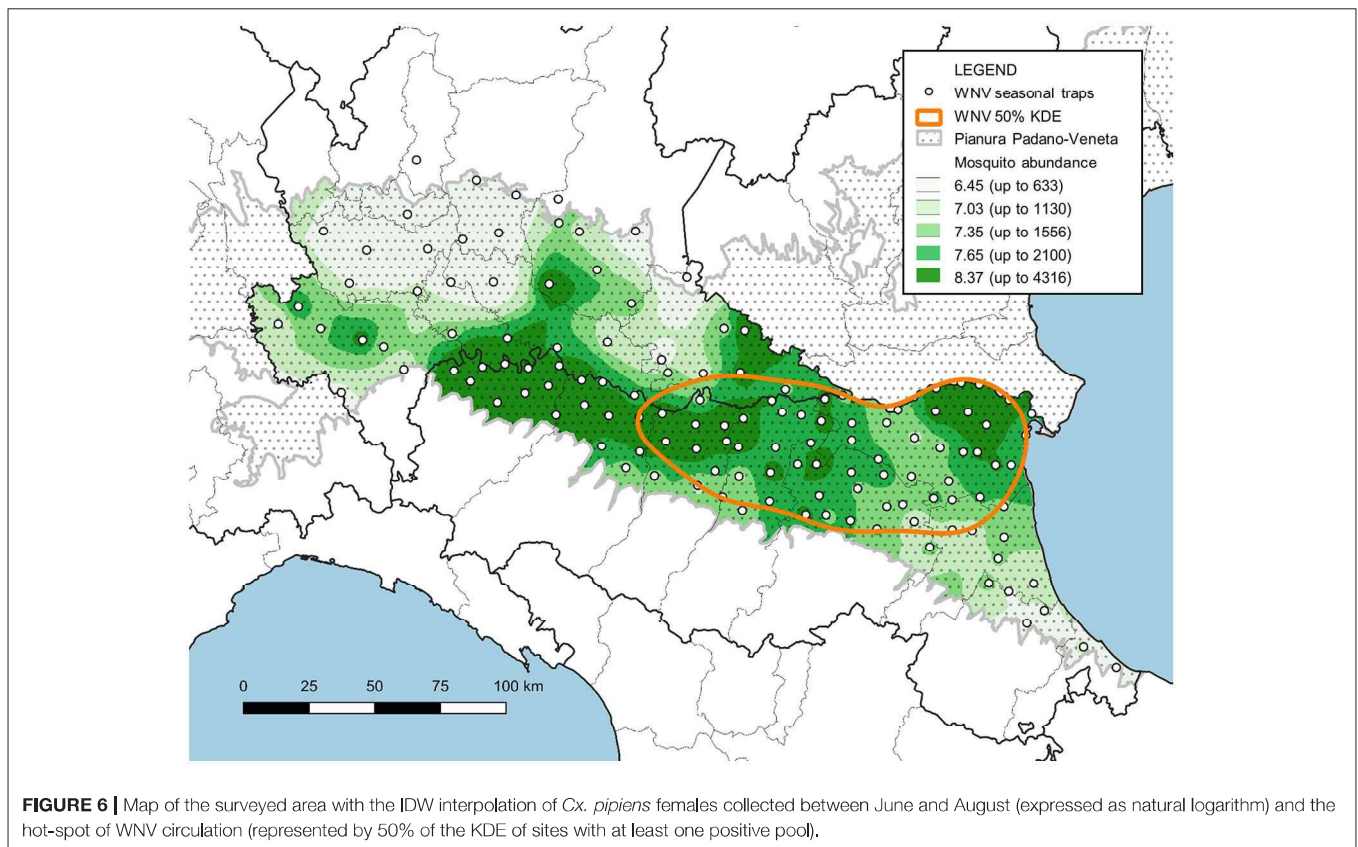


before sampling ( $\beta$  at lag 1 : 0.24, 95% CI 0.16–0.31). In contrast, we did not find evidence of association between the logarithm of the count of sampled mosquitoes and the number of wet days at any lag (Table 5).

When analyzing the proportion of WNV positive mosquitoes, we observed a positive association between the infection rate and the weekly average of maximum temperatures recorded two weeks before the sampling ( $\beta$  lag 2: 0.22; 95% CI: 0.02–0.42) (Table 5). No evidence of association was detected between the infection rate and the weekly number of wet days observed in the previous 4 weeks (Table 6).

## DISCUSSION

In 2018, in Italy as in other European countries (4), the WNV transmission season began earlier than in previous years. In fact, in Emilia-Romagna, the season started earlier by about one month with respect to previous transmission seasons and the Lombardy region. Circulation intensity and the number of human cases were also the highest observed since the first appearance of WNV in Italy. Starting from the central Provinces of Emilia-Romagna (Modena, Bologna, and Ferrara), WNV circulation progressively extended to both



**TABLE 5 |** Linear regression coefficient ( $\beta$ ) and 95% Confidence Interval (CI) for the logarithm of the number of *Culex pipiens* Sampled and Lagged Meteorological Parameters.

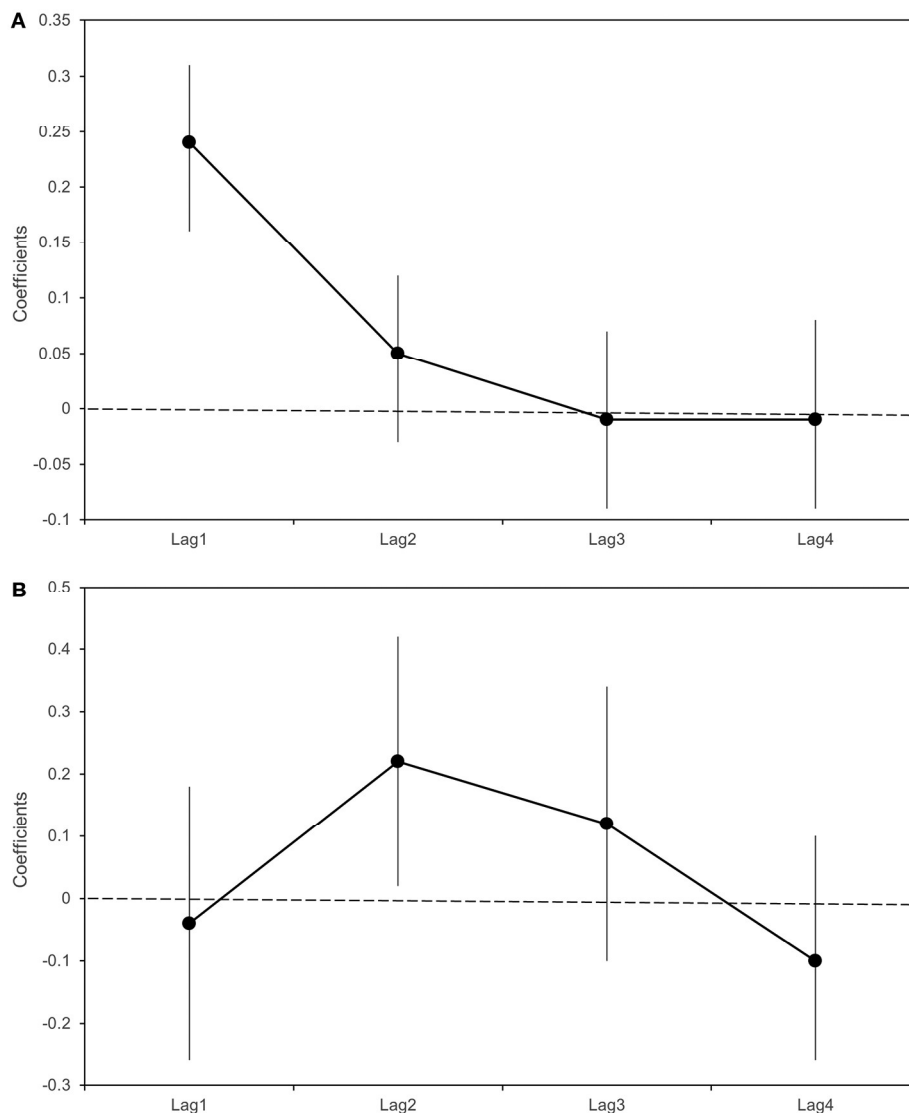
Parameter	$\beta^1$	95% IC
<b>1°C Increase in weekly average maximum temperatures</b>		
Lag1	<b>0.24</b>	<b>0.16 – 0.31</b>
Lag2	0.05	–0.03 – 0.12
Lag3	–0.01	–0.09 – 0.07
Lag4	–0.01	–0.09 – 0.08
<b>1 Day increase in the weekly number of wet days</b>		
Lag1	0	–0.10 – 0.10
Lag2	–0.06	–0.13 – 0.01
Lag3	–0.01	–0.08 – 0.06
Lag4	–0.04	–0.11 – 0.03

<sup>1</sup>Linear regression coefficient ( $\beta$ ): it indicates the average change in the logarithm of *Cx. pipiens* sampled associated with a 1°C increase in weekly average maximum temperatures and 1 day increase in weekly number of wet days. *p* value < 0.05 in bold.

the western and eastern part of the surveyed area covering all the Emilia-Romagna region right up to Lombardy (Pavia, Brescia, Milano, Lodi, and Mantua). However, the virus was undetected in mosquitoes collected in Cremona, Bergamo, Monza Brianza, Varese, and Lecco. The early detection of the virus circulation at provincial level makes it possible

to start test blood bags when an area is affected, avoiding infected transfusions.

Reported data confirmed the *Cx. pipiens* mosquito as the main vector of WNV in the surveyed area. To evaluate the involvement of other species, pools of *Ae. caspius* and *Ae. albopictus* were also tested during this survey, without any positive results. The possible involvement of *Ae. albopictus* as a bridge vector in the WNV cycle was deduced due to WNV positive pools collected in the field in the U.S. (21) and Turkey (22), and the vectorial competence demonstrated in experimental studies (23, 24). The possible vector role of *Ae. caspius*, since WNV-positive pools of this mosquito were collected in the field in the surveyed area (25), still remains to be demonstrated as we have no clues as to these two mosquitoes' involvement in the WNV cycle in our epidemiological scenario. Independently of intrinsic competence, this was likely due to the host preference of these mosquitoes which, contrary to *Cx. pipiens* (strictly ornithophilic), are mammophilic feeders. Also, *Cx. modestus*, a species more competent for WNV than *Cx. pipiens* in a laboratory study (26), had a marginal role in the monitored area's WNV cycle. This is due to the scarcity of this species, which is strictly linked to the natural environment (13). Results of extra-plan samples in some cities highlighted the intensive viral circulation in 2018, also involving urban areas which are usually less suitable than rural areas for WNV circulation. It is likely that virus circulation in urban areas, also sustained by *Cx. pipiens*, had an important role in causing the large amount of human cases recorded in 2018.



**FIGURE 7 |** Beta coefficients (black dots) and 95% Confidence intervals (bars) with respect to a 1°C increase in weekly average maximum temperatures in the previous 4 weeks (lag 1–4). Linear regression estimates for the logarithm of the number of sampled *Culex pipiens* (A); Poisson regression estimates for Infection Rate (B).

The surveillance system showed a relevant difference in WNV circulation intensity between the two regions, which was also confirmed by the difference in the number of human cases. Nevertheless, different mosquito sampling efforts between the two regions, and the difference recorded in the GLM, was largely ascribable to the different circulation extent between the two areas. The applied models demonstrate the final number of infected mosquitoes increases when the number of mosquitoes also increases. However, the probability of a single mosquito being infected decreased with mosquito abundance, revealing a non-linear relationship between mosquito abundance and virus circulation. This result agreed with the absence of a direct correlation between mosquito abundance and virus circulation in years with a lower WNV circulation (7). The geographic interpolation of the data utilized to characterize this relationship

demonstrated that the rate of infected mosquitoes was not higher where mosquito density was highest. This confirms a less than linear correlation between virus presence and mosquito abundance, temporally and spatially.

The data obtained demonstrated the obvious importance of mosquito abundance but highlighted that this is not the limiting factor for virus circulation in the considered area, which we identified as likely in the number of infected hosts. If we postulate the finite number of infected hosts as a limiting factor, and then the finite capacity to attract mosquitoes, we can hypothesize a dilution effect exerted by the mosquitoes not attracted to them, above a certain number of mosquitoes. In this theoretical scenario, the synchronization of mosquito population peaks and WNV-susceptible birds is fundamental in determining WNV dynamics and the environmental viral load for the season. The

**TABLE 6 |** Poisson regression coefficient for Infection Rate and Lagged Meteorological Parameters.

Parameter	$\beta^1$	95% IC
<b>1°C Increase in weekly average maximum temperatures</b>		
Lag1	-0.04	-0.26–0.18
Lag2	<b>0.22</b>	<b>0.02–0.42</b>
Lag3	0.12	-0.10–0.34
Lag4	-0.1	-0.26–0.10
<b>1 Day increase in number of wet days</b>		
Lag1	0.07	-0.17–0.32
Lag2	0.13	-0.06–0.32
Lag3	-0.18	-0.37–0.02
Lag4	0	-0.16–0.16

<sup>1</sup>Poisson regression coefficient ( $\beta$ ): the exponential of  $\beta$  indicates the rate ratio, namely the change in the infection rate (multiplicative term) associated with a 1°C increase in weekly average maximum temperatures and 1 day increase in weekly number of wet days. *p* value < 0.05 in bold.

observed pattern may also support the hypothesis that increased mosquito density leads to increased avian defensive behaviors, leading to a shift in mosquito feedings toward less defensive hosts, such as mammals which are not WNV-susceptible (27, 28).

A complex interaction between mosquito abundance and susceptible hosts determines the level of virus circulation, which differed in direct relation (namely more mosquitoes, more virus) and is usually retained in vector-borne diseases. We recommend that this result is considered in the construction of epidemiological models and in the cost-efficacy analysis of vector control methods.

In this study, temperature was confirmed as one of the most important factors in determining the virus circulation. We detected an association between temperatures recorded one week and two weeks before mosquito sampling and the mosquito infection rate. Temperature influenced the bionomics of mosquitoes in several ways, for example, by increasing vectorial capacity or shortening the extrinsic incubation period and development times (29). These results are consistent with previous studies that observed a positive association between temperatures and WNV-positive mosquitoes and human cases observed in the following weeks (30–32). This association, already confirmed by several research studies in different ecological settings, is alarming in a global warming scenario, since it implies that increasing temperatures will increase the risk of WNV circulation. The same association can partially explain the recent emergence of the virus, which could be linked to the increase in temperatures recorded in recent years in the surveyed area (33). Interestingly, we observed the difference of one or two weeks in the temperature's ability to influence the number of mosquitoes and the virus infection rate. This observed delay could be explained with the virus' extrinsic incubation period, which consistently lasts about one week at temperatures recorded in the surveyed area (34, 35).

As recorded in another study (31), we were unable to find a consistent link between rainfall (expressed as wet days), mosquito abundance and the mosquito infection rate. An issue related to

this process, which can hide the effects of rain, could be the background noise linked to the fall in temperature caused by rainfall. However, water availability under ordinary conditions was not a limiting factor in the study area (due to the widespread presence of irrigating networks, rivers, and water basins).

A human case of WNNND is of striking relevance, due to the severity of the symptoms associated with the infection. However, occurrence is rare and not relevant for the environmental persistence of the virus. Despite this, occurrence of WNNND was the best available indicator of circulation intensity in people, since there was no organic and standardized system for WNV fever diagnosis. The timing and location of WNNND cases are random due to the low rate of WNNND cases in infected persons (<1%), the complexity in defining a certain site of patient infection and has human density as one of the main determinants (36), in a particular area. Despite this, we were able to find a clear relationship between the rate of positive mosquitoes and human cases, highlighting the surveillance ability to assess the intensity of virus circulation in a given area at an early stage.

The entomological monitoring described in this work is part of a multidisciplinary surveillance integrating other monitoring systems (wild bird testing and syndromic surveillance of horses). Entomological surveillance often provided the first sign of WNV circulation and, due to the sampling program's standardized regularity, allowed for a fine characterization of the period, area and intensity of circulation. This provides essential data for modulating actions to limit the health risks associated with the circulation of WNV.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

## AUTHOR CONTRIBUTIONS

MCa, PA, RC, DC, MPC, MCh, GG, MT, RB, and DL conceived and designed the surveillance system. MCa, SC, and TT conducted the sampling and mosquito identification. PB and DT performed the molecular analysis. MCa, GM, LB, and AA processed the data and performed statistical analyses. MCa wrote the manuscript. All authors reviewed and approved the final manuscript.

## FUNDING

Data were obtained within the framework of the Regional Plan for Surveillance and Control of Arboviruses—2018 (Emilia-Romagna Region and the Lombardy Region), and the West Nile and Usutu Viruses Integrated National Surveillance and Response plan—2018. Scientific work was partially financed by IZSLER.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# CHAPTER 5

## Integrating environmental, entomological, animal and human data to model the *Leishmania infantum* transmission risk in a newly endemic area in Northern Italy

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*One Health*, 2020 December; **10**: 100159

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**G. Moirano:** Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft

**S. Zanet:** Data curation (entomological data), Writing – review & editing

**E. Giorgi:** Methodology, Software, Writing – review & editing

**E. Battisti:** Data curation (entomological data), Writing – review & editing

**S. Falzoi:** Data curation (environmental data), Writing – review & editing

**F. Acquaotta:** Data curation (environmental data), Writing – review & editing

**S. Fratianni:** Data curation (environmental data), Writing – review & editing

**L. Richiardi:** Methodology, Writing – review & editing

**E. Ferroglio:** Investigation (entomological data), Writing – review & editing

**M. Maule:** Methodology, Supervision, Writing – review & editing



# Integrating environmental, entomological, animal, and human data to model the *Leishmania infantum* transmission risk in a newly endemic area in Northern Italy

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## ARTICLE INFO

### Keywords:

Leishmaniasis  
Spatial epidemiology  
One health

## ABSTRACT

**Introduction:** Historically, leishmaniasis in Italy was constrained to areas with Mediterranean climate. In the last 20 years, sand fly vectors (*Phlebotomus perniciosus*), cases of canine leishmaniasis (CanL) and cases of human visceral leishmaniasis (VL) have been observed in Northern Italian regions, traditionally classified as cold areas unsuitable for sand fly survival.

**Aim:** We aim to evaluate through a One-Health approach the risk of endemic transmission of *Leishmania infantum* in the Piedmont Region, Northern Italy.

**Methods:** We collected environmental, entomological, animal, and human data. We applied a geostatistical binomial model to map the probability of *P. perniciosus* presence in the study area, using selected environmental parameters as predictors. We evaluated the spatial relationship between the probability of *P. perniciosus* presence and the geographical distribution of CanL and VL cases observed between 1999 and 2013.

**Results:** Between 1999 and 2003, 142 sampling sets (17%) out of 839 resulted positive for *P. perniciosus*. Elevation, degree of slope, normalized difference vegetation index (NDVI) and summer temperatures were associated with positive sampling sets. During the study period, 164 (13.6%) of Piedmont municipalities reported at least one autochthonous case of CanL, while 89 VL cases were observed in 54 municipalities (4.5%). We observed an association between municipalities affected by autochthonous CanL cases and the estimated probability of *P. perniciosus* presence (Odds Ratio for 10% increase of probability: 2.66; 95% confidence intervals (CI): 2.16–3.37). We found that human VL incident cases were positively associated with the probability of the municipality of residence of being endemic for CanL (Incidence Rate Ratio for 10% increase of probability: 1.49; 95% CI 1.02–2.16).

**Conclusions:** Using a One-Health approach, we quantified the spatial association between the distribution of *P. perniciosus*, municipalities endemic for CanL and incident cases of human VL, suggesting that the disease has become endemic in the Piedmont region.

## 1. Introduction

Human visceral leishmaniasis (VL) is a vector-borne disease caused by a parasite of the *Leishmania* genus [1]. In the Mediterranean basin, *Leishmania infantum* represents the main putative agent of VL [2]. The parasites are transmitted to susceptible organisms, such as humans and dogs, by the bite of female phlebotomine sand flies [1]. Human VL is

usually diagnosed clinically among subjects that present irregular fever, anaemia, leukopenia and hepatosplenomegaly. Whereas most immunocompetent individuals will not develop any symptoms after *Leishmania* infection, immunocompromised subjects develop a VL, which is typically fatal if untreated [3]. In the canine host, *L. infantum* causes the canine leishmaniasis (CanL), a severe disease that occurs in fewer than 50% of infected animals. Both symptomatic and

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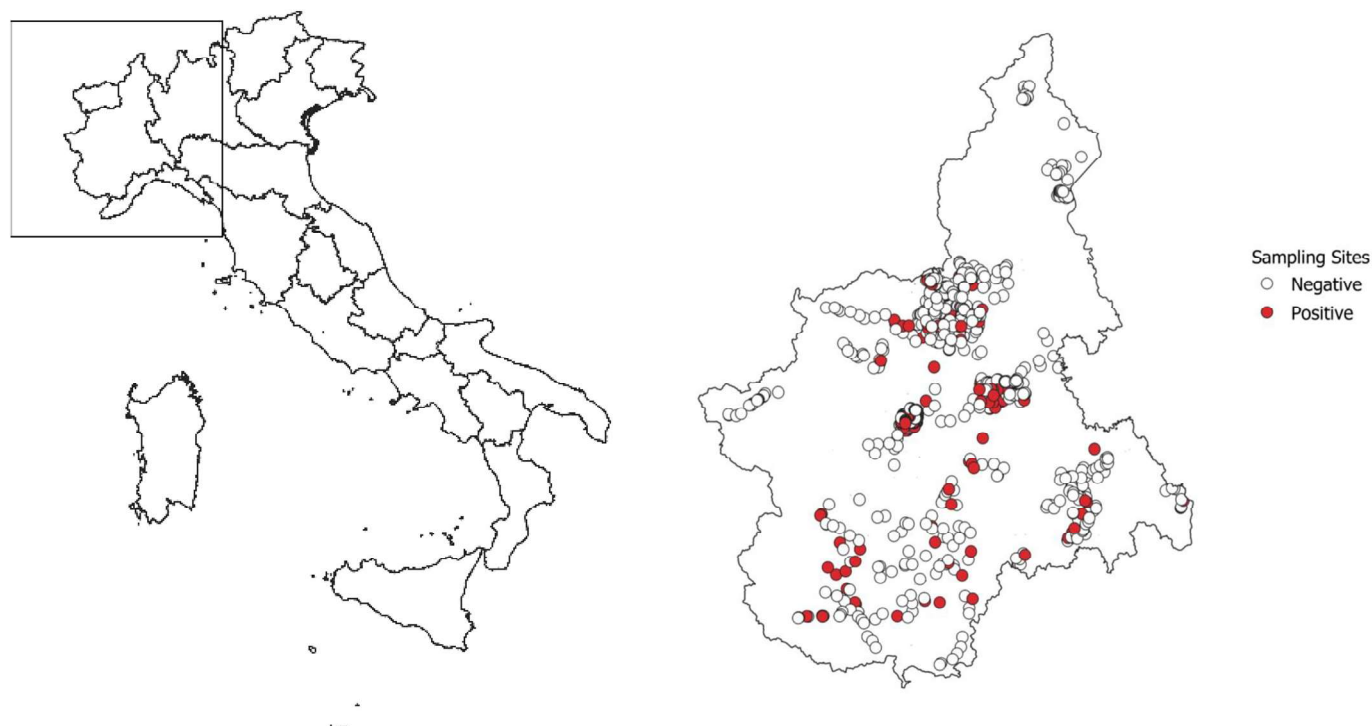
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<https://doi.org/10.1016/j.onehlt.2020.100159>

Received 23 April 2020; Received in revised form 6 August 2020; Accepted 6 August 2020

Available online 26 August 2020

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**Fig. 1.** Study area and distribution of sampling sets.

Left Panel: the framed area includes the study area. Right panel: *P. perniciosus* sampling sites. White dots: negative sampling sets, red dots: positive sampling sets. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

asymptomatic infected dogs also act as reservoir hosts for the human disease [4].

Transmission of *L. infantum* is associated with the geographical distribution and abundance of competent vectors, mainly represented in Italy by *Phlebotomus perniciosus* [5,6]. The geographical distribution of phlebotomine sand flies is affected by several environmental parameters, such as altitude, land-cover, vegetation, air temperature and relative humidity [7,8]. For instance, air temperature is believed to influence both the vector distribution and the seasonal activity of the sand flies [9,10]. Consistently, cases of both canine and human leishmaniasis have been historically reported in central and southern peninsular Italian regions, characterized by hot summers and mild winter temperatures [11,12]. However, in the last 20 years, both sand fly vectors and cases of CanL and human VL have been observed in Northern Italian regions, traditionally classified as colder areas characterised by continental climate [13].

Assessing and measuring the endemic status of human VL in recently affected areas, such as Northern Italy, raises difficulties. Due to the long VL incubation period and the possibility of frequent travels to endemic areas (such as trips to the Mediterranean coastal areas of Northern Italy), the occurrence of human cases in non-endemic regions could be an unreliable indicator of new endemicity. On the contrary, assessing the geographical distribution of the vectors and canine cases does not directly allow evaluation of the transmission risk to human populations. For these reasons, a One-Health approach, integrating environmental, entomological, animal, and human data has been proposed as a valid option to study and control the transmission of Leishmaniasis [14,15]. With this study, we have evaluated, through a One-Health approach, the risk of transmission of *L. infantum* in the Piedmont region, an area of northern Italy with an increasing number of human VL cases [13,16].

## 2. Methods

### 2.1. Study area

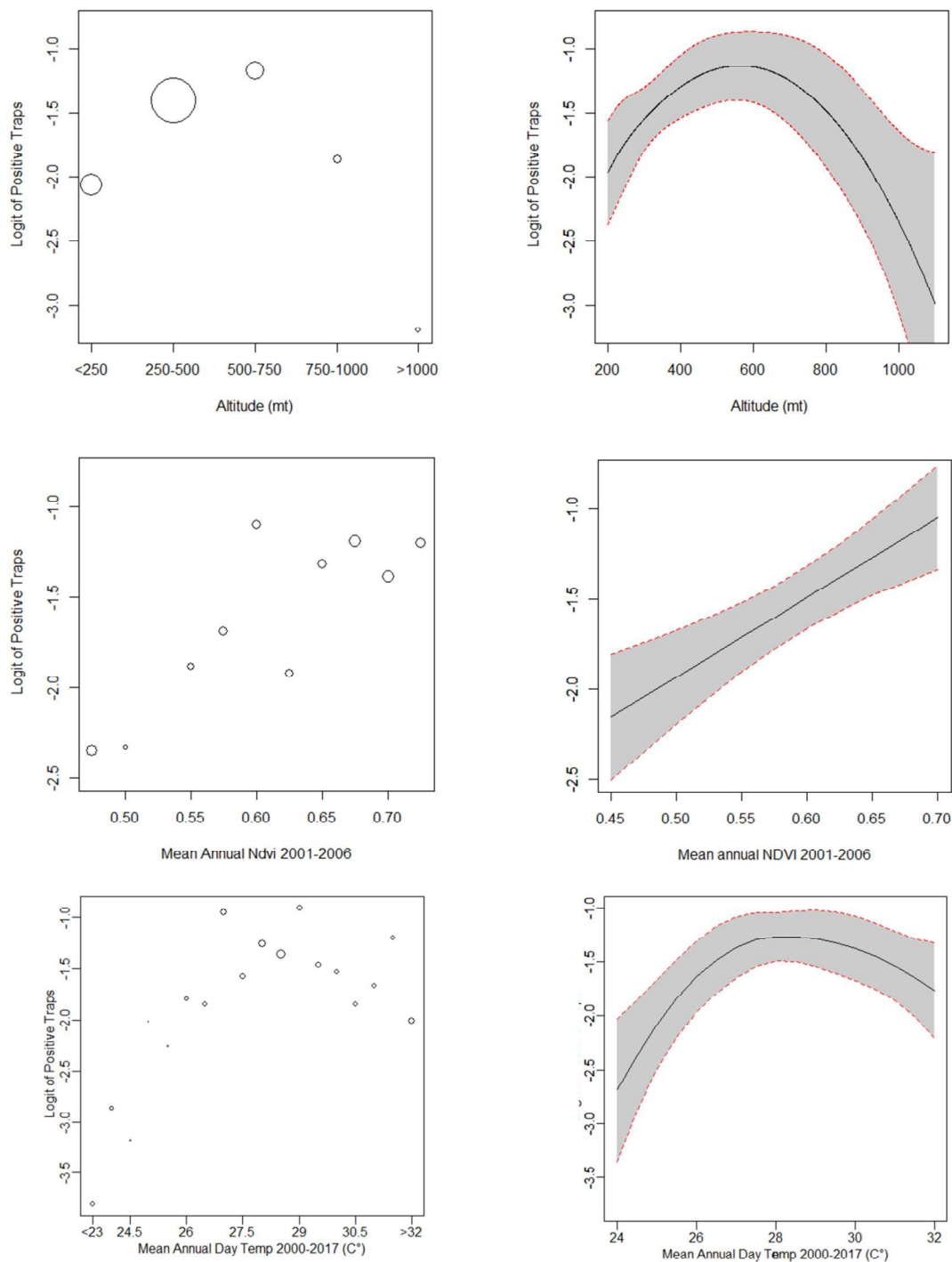
Piedmont is a region in the north-west of Italy with a surface of 25,387 km<sup>2</sup>. The altitudinal range varies from 4632 m a.s.l. of the highest peak to 72 m a.s.l. of the Padan Plain. Piedmont surface topography is characterized by 43% mountains, 30% hills and 26% plains. According to the Köppen and Geiger classification, in plains and hilly areas the climate is continental temperate or subcontinental temperate with hot summers and generally cold winters [17]. However, the area presents different climatic features because of the presence of several microclimates determined by the presence of the Alps mountains, hills, the Padan Plains and Lake Maggiore. The air temperatures show a regular decrease with elevation, except for changes due to geographical setting [18].

The region is divided in 1206 municipalities and 8 provinces and has roughly 4.3 million inhabitants. The population density is on average 174 inhabitants per km<sup>2</sup>, but is highly heterogeneous, reflecting the orography of the area. Population density steadily increases from low densely inhabited mountainous areas, to hilly areas, and then to the densely inhabited Padan Plains, reaching its peak in the urbanized area of the Metropolitan City of Turin.

### 2.2. Data collection

#### 2.2.1. Entomological data

We obtained information about the presence/absence of *P. perniciosus* from 839 sampling sets randomly identified from potentially suitable areas across Piedmont Region between 1999 and 2003. In total 208 out of 1206 municipalities (23%) of Piedmont region were covered by the sampling activities. Briefly, each sampling set consisted in 10 to 20 sticky traps (total area: from 0.4 to 0.8 m<sup>2</sup>) embedded with castor oil placed in suitable sand fly diurnal resting sites (animal shelters, houses, and scarp wall cracks) for one night, from sunset to sunrise [7].



**Fig. 2.** Proportion of positive sampling sets and continuous environmental predictors (*altitude, NDVI, temperature*).  
 Upper Panel: Fig. A: Logit transformation of proportion of positive sampling sets in relation to elevation, size of circle is proportional to the number of observations in the range shown in the x axis. Fig. B: Fitted value of Logit transformation of proportion of positive sampling sets and 95% CIs.  
 Central Panel: Fig. A: Logit transformation of proportion of positive sampling sets in relation to NDVI, size of circle is proportional to the number of observations in the range shown in the x axis. Fig. B: Fitted value of Logit transformation of proportion of sampling sets and 95% CIs.  
 Lower Panel: Fig. A: Logit transformation of proportion of positive sampling sets in relation to summer temperatures (average 2000–2017), size of circle is proportional to the number of observations in the range shown in the x axis. Fig. B: Fitted value of Logit transformation of proportion of sampling sets and 95% CIs.

Sampling activities were performed during the summer months (from the second half of June to the first half of August) when the highest sand fly densities are expected [9]. Collected insects were stored in 70% ethanol until further analysis. All specimens were cleared in chloralactophenol and mounted in Hoyer medium and morphologically identified according to taxonomical keys [19,20].

### 2.2.2. Environmental data

We selected four environmental predictors potentially associated with the presence of *P. perniciosus* from the literature [5,7,9,10,21], namely elevation, slope, normalized difference vegetation index (NDVI) and summer maximum temperatures. Data about elevation were obtained from the Digitalized Elevation Model (DEM) raster with a resolution of  $1 \times 1$  km released by the Italian Institute of Environmental

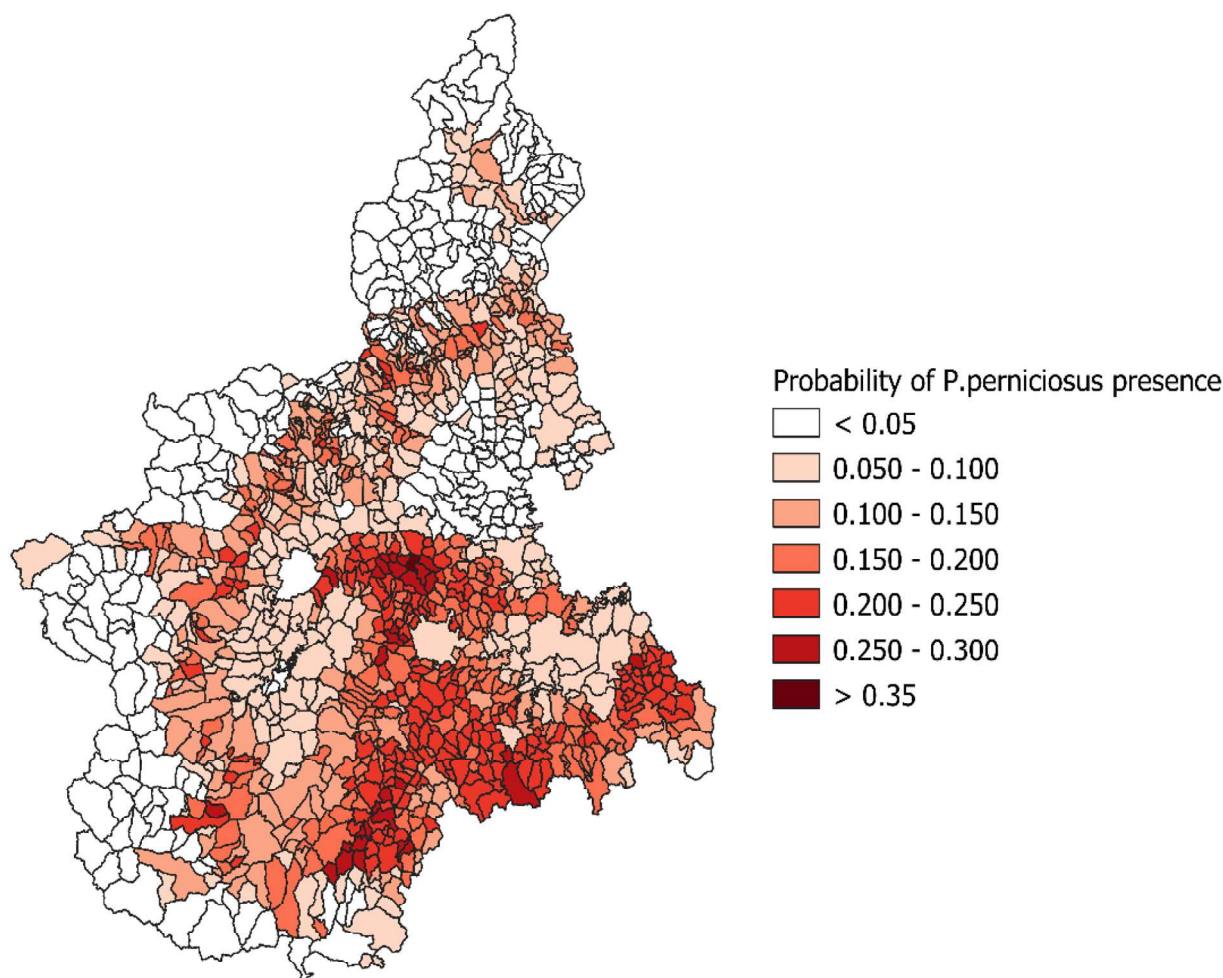


Fig. 3. Geographical distribution of the estimated probability of the presence of *P. perniciosus* aggregated at the municipality level.

Protection and Research (ISPRA) [22]. Slope was computed from the DEM raster in GIS environment and was expressed in degrees to describe the angle of inclination of the terrain. The NDVI annual average values in the period 2001–2005 were computed using data from the Moderate Resolution Imaging Spectroradiometer (MODIS) Terra satellite with  $1 \times 1$  km resolution and used as a proxy of greenness. Monthly average temperatures for the period 2001–2017 were obtained from MODIS Land Surface Temperature (LST) with an approximate resolution of  $1 \times 1$  km. The monthly daytime mean temperatures for June, July and August were averaged and used as a proxy for the daytime summer temperatures.

### 2.2.3. Canine data

For each municipality of the Piedmont region, we obtained information about the presence or absence of detected autochthonous cases of CanL between 1998 and 2013. Data were retrieved from an online map produced within a study conducted by the National Institute of Health. In this study, municipalities were classified in relation to the canine leishmaniasis as “disease free” or “affected” [23]. Presence of endemic CanL was assessed by testing with the Indirect Fluorescent Antibody Test (IFAT) serum samples of dogs that were likely to be infected or sick and that had not travelled to endemic areas.

### 2.2.4. Human data

The Hospital Discharge Register (HDR) records were used to identify incident cases of VL among residents of the Piedmont region, using the International Classification of Diseases, 9th Revision, Clinical Modification (ICD 9 CM) codes. Hospital discharges for VL (ICD 9

CM = 085.0 in any diagnostic position) recorded between January 1, 1995 and December 31, 2013 (19 years) were selected. In this database, each hospitalized patient is identified by an anonymous ID code. Patients with VL can be readmitted to the hospital multiple times, since disease relapses are frequent. We used the ID code to identify, for each patient, only the first VL-related hospitalization and to exclude readmissions. Since VL can last many months, we introduced a lag time of 4 years to define incident cases. By introducing a 4-year lag, we identified incident cases for the period 1999–2013 (15 years) excluding subjects that had been already admitted for VL between 1995 and 1998. In addition, we restricted our study to Italian citizens. In the study area, indeed, VL is a rare disease and almost all identified VL cases with foreign nationality were natives of countries where VL is endemic. To perform spatial analysis, cases were attributed to their residential address at the time of hospitalization. Population data for the Piedmont region were obtained from the Italian National Statistical Institute (ISTAT) [24].

## 2.3. Statistical analysis

### 2.3.1. Geostatistical modelling of the presence of *P. perniciosus*

An initial exploratory analysis was carried out to assess the relationship between the environmental predictors and the proportion of traps positive for *P. perniciosus*. Through a multivariable logistic regression, we modelled the association between selected environmental predictors and the proportion of traps positive for *P. perniciosus*. Based on the results of an exploratory analysis, the effect of NDVI was modelled with a linear function, while the effect of elevation and summer

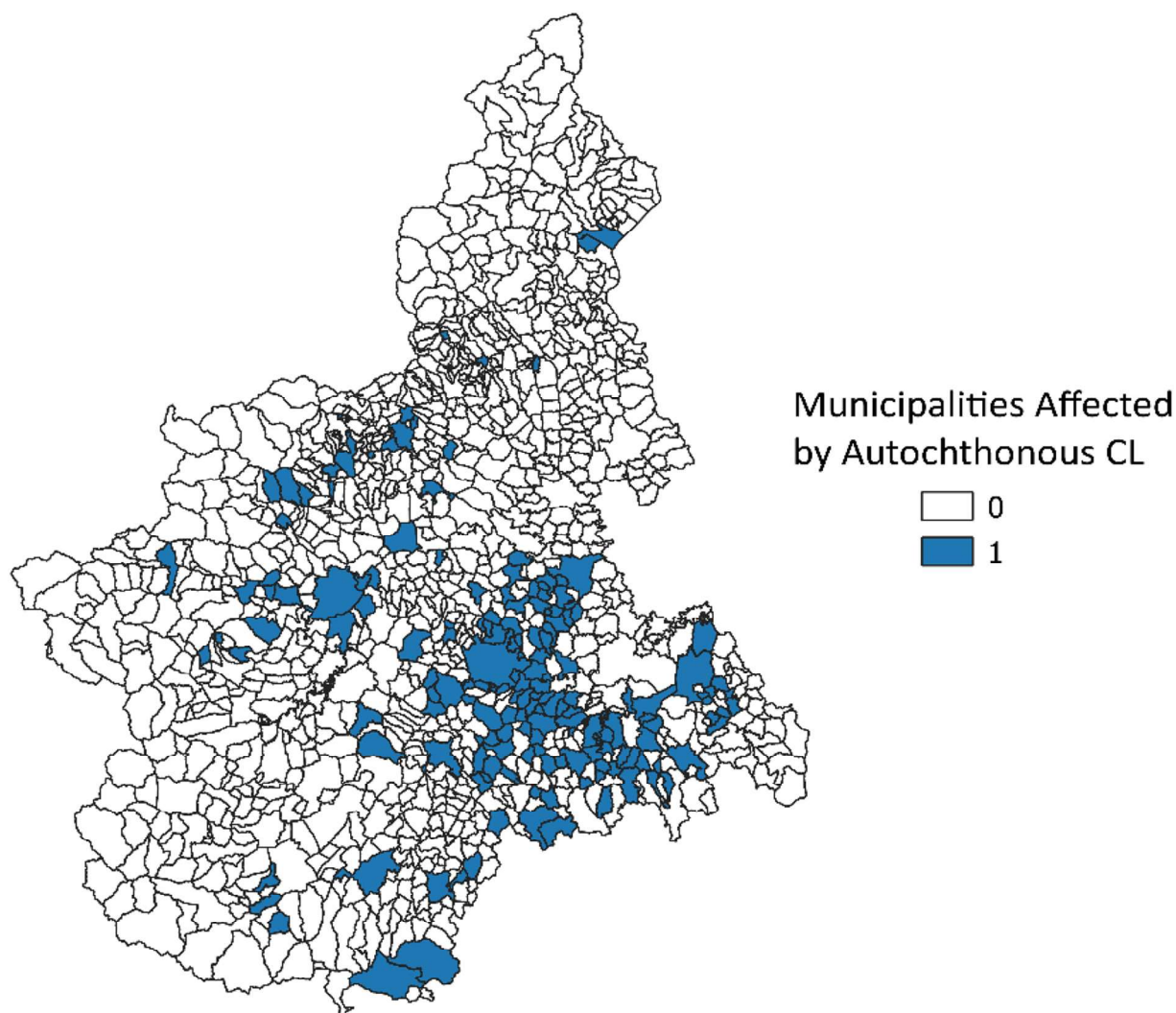


Fig. 4. Geographical distribution of the municipalities affected by autochthonous CanL cases.

temperatures were modelled through quadratic splines. Slope was dichotomised into flat (slope < 2°) and steep areas (slope ≥ 2°). Scatter plots and equations included in the final model are reported in the supplementary materials. In the final multivariable analyses, geostatistical binomial regression with a logit-link function was used to model the proportion of positive sampling sets. In order to lower the computational burden of the geostatistical models, sampling sites located less than 250 m from each other were attributed to the same sampling site. More specifically, the proportion of positive sampling sets ( $N = 839$ ),  $p(x)$ , at site  $x_i$  ( $N = 749$ ) was modelled as:

$$\log\left(\frac{p(x_i)}{1 - p(x_i)}\right) = \alpha + \beta d(x_i) + S(x_i)$$

where:  $d(x)$  is the vector of the georeferenced explanatory variables;  $S(x)$  is a random effect that accounts for the spatial correlation between observations induced by unmeasured factors affecting the presence of *P. perniciosus*. In this case,  $S(x)$  was modelled as a zero-mean stationary and isotropic Gaussian process with spatial correlation function given by:  $\text{Cor}\{S(x), S(x')\} = \exp(-||x - x'||/\phi)$ . Where  $\phi$  is the scale parameters which regulates the rate of decay of the spatial correlation for increasing distance, and  $||x - x'||$  is the distance in space between the two spatial points  $x$  and  $x'$ . From the fitted geostatistical model, we plotted a predictive map of the probability of the presence of *P. perniciosus* over a  $1 \times 1$  km grid. The gridded probability was aggregated at the municipality level to evaluate its relationship with the presence of

VL and CanL cases. Given that the inhabitants are not distributed homogeneously within a municipality area, we computed the population-weighted average of the estimated gridded probability, using a population density raster with  $1 \times 1$  km resolution [25]. Parameter estimation and spatial prediction were carried out using the PrevMap package in the R software environment [26]. Maps presented were created using Quantum GIS 3.4.

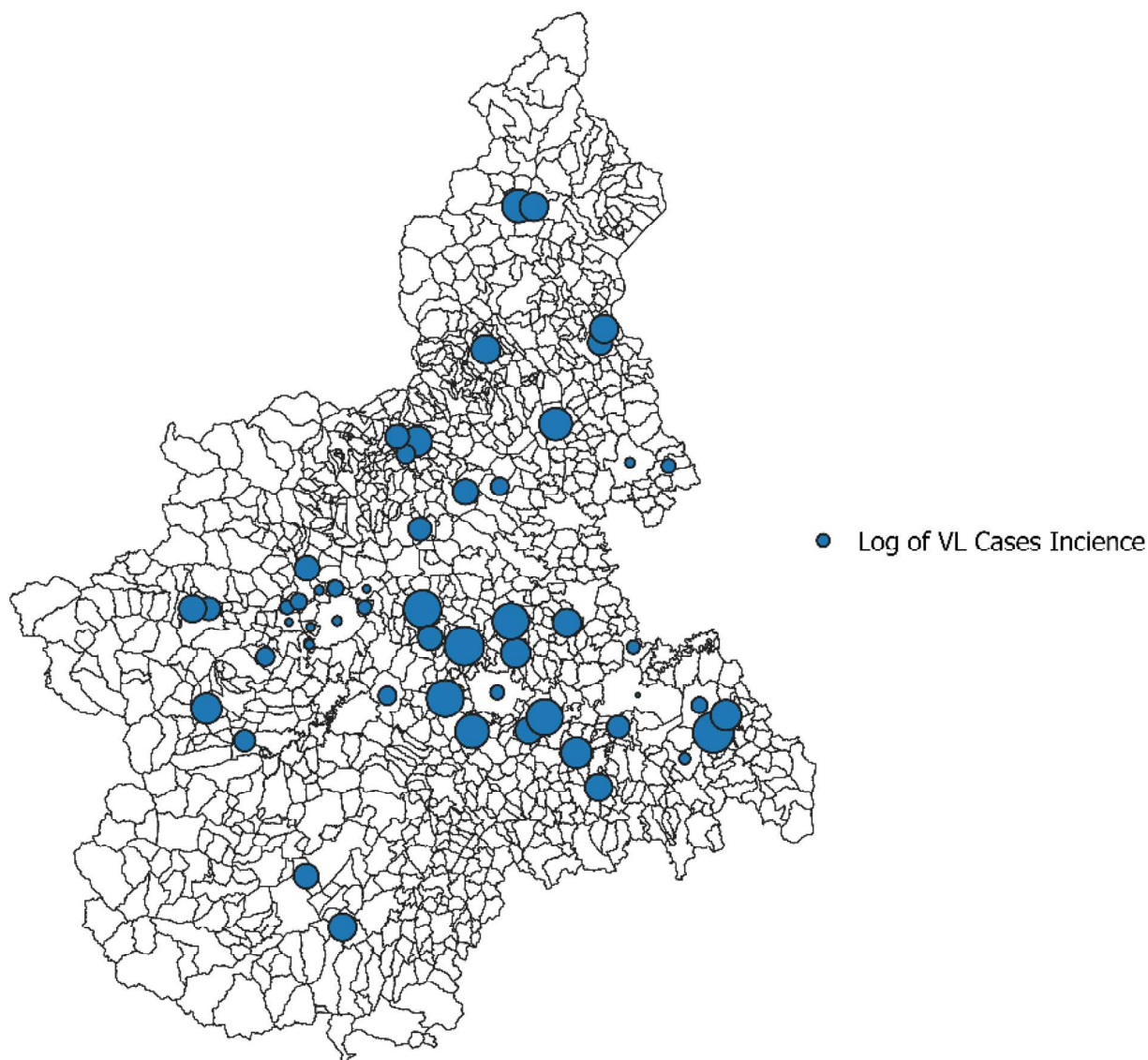
### 2.3.2. Statistical modelling of human VL cases, endemic CanL and *P. perniciosus* presence

For each municipality, we studied the association between the estimated probability of *P. perniciosus* presence and the endemic status for CanL. We fitted a logistic regression model with  $CanL_m$  (0: municipality free from CanL; 1: municipality with autochthonous cases of CanL) as the outcome and  $p(Phl_m)$  (predicted probability of *P. perniciosus* presence at the municipality level) as the explanatory variable:

$$\text{Model 1} \quad \log\left(\frac{p(CanL_m)}{1 - p(CanL_m)}\right) = \alpha + \beta * p(Phl_m)$$

From the fitted model we estimate  $p(CanL_m)$ , namely the probability of each municipality of being endemic for CanL given the *P. perniciosus* presence.

To model human cases, we used a negative binomial regression. We modelled age- (0–25, 25–75, > 75 years) and sex-standardized incidence rates of VL cases at the municipality level, computing the



**Fig. 5.** Geographical distribution of human VL incident cases by municipality.

All cases recorded in a given municipality have been geographically attributed to the centroid of the municipality of residence. Dot size is proportional to the log incidence rate at the municipality level.

expected incident cases for each stratum. We fitted a negative binomial regression to incident cases at municipality level, using  $p(\text{CanL}_m)$  as the explanatory variables:

$$\text{Model 2 } \log(\mu_m) = \alpha + \beta * p(\text{CanL}_m) + \log(E_m)$$

where  $\mu_m$  and  $E_m$  are respectively the observed and the expected number of human VL cases at municipality level, while  $p(\text{CanL}_m)$  is the estimated probability for each municipality of being endemic for CanL estimated from Model 1.

### 3. Results

Between June 1999 and September 2003, 839 sampling sets in the study area were tested (Fig. 1). Overall, 142 sets (17%) resulted positive for *P. perniciosus*. All environmental predictors resulted associated with the probability of positive traps sets. The relationships between the proportion of positive sets and elevation, slope, NDVI and summer temperatures are shown in Fig. 2. At the municipality level, the median value of the estimated probability of the presence of *P. perniciosus* was 11.3% (5th–95th centiles: 0.3%–30.5%). The geographical distribution

of the estimated probability is shown in Fig. 3. The highest probability was observed in the central hilly area of the region and in the pre-Alps, while the lowest probability was predicted in the mountain areas and in the eastern Padan Plains. Between 1998 and 2013, 164 municipalities of the Piedmont region (13.6%) reported at least one autochthonous case of CanL (Fig. 4). Between 1999 and 2013, 89 human VL cases were observed in 54 municipalities (4.5%) of the study area (Fig. 5). At the municipality level, we observed a positive association between the presence of autochthonous CanL cases and the estimated probability of *P. perniciosus* presence (OR for 10% increase of  $p(\text{Phl}_m)$ : 2.66; 95% CI: 2.16–3.37). When we fitted the negative binomial regression to VL cases, we found that the incident cases were positively associated with the probability of residence of being endemic for CanL (IRR for 10% increase in  $p(\text{CanL}_m)$ : 1.49; 95% CI 1.02–2.16) (Table 1).

### 4. Discussion

The Piedmont Region, north-west Italy, was not previously considered endemic for *L. infantum*, but recent studies have documented

**Table 1**

Association between the probability of the presence of *P. perniciosus*, the presence of canine leishmaniasis and human visceral leishmaniasis in Piedmont municipalities.

Model 1: logistic regression			
Outcome	Exposure	OR	95% CI
CanL <sub>m</sub>	p(Phl <sub>m</sub> )	2.66	2.16–3.37
Model 2: negative binomial regression			
Outcome	Exposure	IRR	95% CI
μ <sub>m</sub>	p(CanL <sub>m</sub> )	1.49	1.02–2.16

OR: Odd Ratios, CI: Confidence Intervals, IRR: Incidence Rate Ratio, CanL<sub>m</sub>: Municipalities affected by autochthonous CanL cases; μ<sub>m</sub>: Incident VL cases at the municipality level; p(Phl<sub>m</sub>): estimated probability of *P. perniciosus* presence aggregated at the municipality level (expressed as 10% increase); p(CanL<sub>m</sub>): probability of a municipality of being endemic for CanL (expressed as 10% increase).

new foci of infection [13,27,28]. With the current study, we used a One-Health approach, integrating environmental, entomological, animal and human data, to systematically assess and map the risk of endemic transmission of *L. infantum* in that region, covering a 15 years timespan.

Results provided by the geostatistical prediction of *P. perniciosus* presence showed a heterogeneous distribution of the vector in the study area. The vector distribution was limited to specific areas, characterized by suitable environmental features. Altitude is one of the most important factors influencing the distribution of *P. perniciosus* [5]. In the present study, the probability of observing *P. perniciosus* resulted higher among hilly and premountainous areas (elevation range 250–750 m) and lower in high mountainous areas (elevation > 1000 m) and in flat areas of the Padan Plains, in line with previous findings [5]. In addition, we observed that the proportion of positive traps sets for *P. perniciosus* was higher in areas characterized by warmer summer temperatures and higher NDVI, which is a proxy of vegetation cover. Summer temperatures greatly influence the ability of the insect to fulfil the lifecycle during the activity season and the *L. infantum* development within the vector. The highest probability of the presence for *P. perniciosus* was found in areas with temperature ranges between 26 and 32 °C, confirming previous experimental and field data [5,29]. The positive association between *P. perniciosus* presence and vegetation cover, described by NDVI in the model, could be explained by the higher presence of organic material that ultimately represents a suitable site for winter larval survival and development. Results provided by the geostatistical model are also consistent with the geographical distribution of canine and human cases. We observed a positive association between municipalities affected by autochthonous CanL cases and the estimated probability of *P. perniciosus* presence. These results were further strengthened by the positive association observed between the incident VL cases and the geographical distribution of CanL. Taken together, these results show that the transmission of Leishmaniasis has become endemic in Piedmont in recent years.

The main limitation of the current study is that the entomological samples are based on monitoring activities performed between 1999 and 2003, while data about CanL cases and VL cases are referred to a timespan that runs until 2013. It cannot be excluded that the geographical distribution of *P. perniciosus* might have slightly changed after sampling, however it is likely that this change has not altered sensibly the geographical heterogeneity of *P. perniciosus* presence in the area. Saying it another way, areas that presented a higher presence of *P. perniciosus* during the sampling period are likely to have kept a higher probability also in the following period.

The main strength of our study lies in the integration of several

sources of information, including environmental, entomological, animal and human data, using a One-Health perspective to evaluate the risk of endemic transmission of *L. infantum* in the Piedmont region. We found positive associations between the spatial distribution of the vector, infected reservoir hosts and human cases. These findings indicate that *L. infantum* strains have been circulating in some areas of the region during the study period. Our results also suggest that it is likely that a proportion of the 89 VL cases observed in Piedmont between 1999 and 2013 have contracted the infection in Piedmont, in contrast to the hypothesis that the majority of VL cases reported in Piedmont region are imported from neighbouring areas, such as coastal areas, historically considered endemic for *L. infantum*. The observation of the occurrence of human VL cases alone would not have been sufficient to identify areas at high risk of endemic *L. infantum* transmission, given the low occurrence of human cases in the study area. On the other hand, our study suggests that, since the majority of VL cases occur in areas where autochthonous cases of CanL are observed and infected dogs are easier to identify (up to 50% of infected dogs develop symptoms [30]), monitoring canine cases of leishmaniasis together with the sampling of sand fly may be a useful tool for public health authorities to identify high-risk areas.

The emerging transmission of *L. infantum* in new affected areas could be explained by the introduction of infected dogs from endemic areas, the expansion of the vector distribution, or the combination of the two [2]. It has been argued that the geographical distribution of Leishmaniasis and its vectors in Europe has been changing over the last decades [8,31,32] and, in particular, that the recent northward spread might be a consequence of the increase in temperatures values and extreme climate indices driven by climate change [33]. Climate change might affect leishmaniasis distribution by the effect of air temperatures on the survival and abundance of its vector species. For instance, recent studies have predicted that climate change could trigger the spread of sand fly vectors in areas outside the present geographical range and lead to an increased risk of contracting leishmaniasis for the human population in several areas of the world [6,34,35]. Our study cannot directly elucidate whether the endemization process observed in the study area is attributable to climate change. However, the expansion of the vector distribution induced by climate change remains one plausible mechanism to explain the northwards shift of *L. infantum* observed in Italy. Cold temperatures play a fundamental role in limiting the geographical distribution of the parasite and its vectors [6,34,35] and continental winters might have prevented the Piedmont region from the endemization of *Phlebotomus* species until the end of the last century. Piedmont, however, in the last decades experienced a non-negligible increasing trend in temperatures, with an increase of 2.3 °C in the average maximum temperatures and an increase of 1.5 °C in the average minimum temperatures in the last 60 years [36]. Observed increasing temperatures could have triggered the expansion of sand fly vectors starting from the end of the last century in Piedmont. To verify this hypothesis, repeated sand fly sampling in old and new sites in the coming years may clarify if and how the geographical distribution and abundance of this vector is increasing and may help to identify the main drivers of such a change.

In conclusion, the current study has shown the use of a One-health approach to identify the geographical distribution of endemic transmission of *L. infantum* in recently affected areas. The results support the hypothesis that an endemization process for *L. infantum* has occurred over the last two decades in the Piedmont region and can be helpful in addressing public health interventions to control disease transmission in emerging high-risk areas, where both human and canine cases occur.

## Funding

None.



## Declaration of Competing Interest

None.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.onehlt.2020.100159>.

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### 6.1 Data, Study Designs and Methods

This research project aimed to explore the role of environmental, meteorological and climatic parameters in shaping the epidemiology of vector borne diseases in Italy through a One-Health Approach.

Obtaining, incorporating and merging different types of data is one of the major challenges that One Health projects face. Since typical datasets usually describe only one aspect of the One-Health domain, the interrogation of several databases is necessary for the generation and testing of research hypotheses. Another challenge comes from the fact that each data collection is generally characterized by specific spatial and temporal resolutions. The environmental variables collected in this project range from land-based meteorological data specific for a single location (Chapter 3) to gridded environmental satellite data (Chapter 4 and 5). Entomological data for WNV modelling include information on vector density and vector infection rate at a weekly scale (Chapter 4), while data about the presence/absence of *P. perniciosus*, the main vector of Leishmaniasis, were collected at a seasonal scale (Chapter 5). Also epidemiological data were obtained from different sources: yearly cases of West Nile Neuro-invasive Disease were retrieved from reports released by the National Institute of Health (Chapter 2), weekly cases of West Nile Virus Infection were obtained from The European Surveillance System (TESSy) (Chapter 3), while hospitalized subjects with a diagnosis of Visceral Leishmaniasis were identified from the Hospital Discharge Registries (Chapter 5). This research project has thus involved the integration and analysis of data obtained from several and heterogenous sources of information.

For instance, in Chapter 2 we aimed to describe the geographical distribution of WNV across Italian regions. Given that different surveillance systems rule endemic and non-endemic regions, part of the spatial heterogeneity of WNV cases could be explained by a greater ease of diagnosis of asymptomatic or mild symptomatic subjects in endemic areas (e.g. through active testing of blood donors) than in non-endemic areas. This guided our choice to limit our analysis to cases of West Nile Neuro-invasive Disease (WNND). The diagnosis

of WNND cases is based on the development of neurological symptoms and requires an etiological diagnosis also in non-endemic areas. Therefore, restricting the analyses to WNND cases lowered the possibility of a reporting bias due to the different surveillance systems. On the other side, in Chapter 3 we were interested in evaluating the temporal association between meteorological variables and the risk of contracting West Nile Virus. We therefore selected all incident cases of West Nile Virus Infection (including cases of West Nile Neuro-invasive Disease, cases of West Nile Fever and asymptomatic subjects tested positive for WNV). The latter outcome is more inclusive and less specific than the outcome used in Chapter 2 but it was the only one available at a temporal scale (weekly) fine enough to design a case cross-over study to assess the temporal association between meteorological variables and WNV risk. Using all incident cases of WNV infection has not allowed the identification of potential differences in the lag-effects between meteorological variables and WNV infection risk among different groups (WNND cases, WNF cases and asymptomatic subjects). It can be hypothesized that the reason of a WNV diagnosis, which depends on the level of symptoms, may be more or less close to the date of infection and, thus, have an influence on the lagged effect of the meteorological variables. The reason of WNV diagnosis, however, was not available at the individual level, thus hampering a stratified analysis.

In addition, the research project embraces different epidemiological study designs. Epidemiological study designs and statistical analysis proposed in this research fall into the general family of spatio-temporal models, usually applied in environmental epidemiology and in medical geography. The choice of a specific epidemiological study design and, consequently, of related statistical analysis was motivated by the hypotheses under study, by the data availability and by the structure of identified datasets.

In our study on the temporal and geographical distribution of the West Nile Virus in Italy (Chapter 2), we had obtained information about the yearly cases of Neuro-invasive Disease aggregated at the province level. We thus attempted to detect spatial-temporal clusters of new cases through a recent method developed for the analysis of aggregated data. The proposed method is based on multiple Maximum Likelihood Ratio Tests corrected for multiple comparison. A possible alternative method was the spatial scan statistic (SSS), which is the most widely used method in epidemiology for cluster detection. However, the method applied in our study is easily implementable and has

been shown to provide similar results of SSS with a lower computational demand.

The analyses for the case-crossover study on WNV in relation to meteorological factors (Chapter 3) were based on a distributed lag nonlinear model (DNLMs). The DNLMs are widely used in the analysis of the association between temperature and mortality. Overall, the main novelty of our study consisted in applying the case-crossover design in the infectious disease context. In our model specification, we assumed linear effects for meteorological variables, modelled the lagged effect with a spline, and then built a tensor product of the two terms. The statistical selection of the best tensor product to describe the relationship between the exposure, the outcome and the lagged effect was based on the Akaike Information Criterion. Another novelty of our study was the inclusion of a spline function in the DNLM to adjust for residual seasonality, which might not be captured by the time-stratified case crossover built on weekly incident cases. Unfortunately, we had information on the week but not on the day of diagnosis and, thus, we could not date back the exposure starting from the day of symptoms onset, but only from the week preceding the week of the diagnosis. However, this limitation is common to most of environmental studies conducted on infectious diseases, since surveillance systems for communicable diseases typically notify cases on a weekly scale.

The approaches used for the analyses in Chapter 4 include different statistical methods, from simple descriptive indexes to more complex models as Generalized Linear Mixed Models (GLMMs). In this study, we attempted to estimate the association between two entomological parameters (namely, vector density and infection rate) and the meteorological parameters recorded up to 4 weeks (lag1–4) before the night of sampling. Opting for GLMMs was motivated by the spatio-temporal structure of the dataset which included repeated measurements in different geographical locations. GLMMs allow to model location-specific and week-specific random effects that can account for spatial and temporal heterogeneity and provide better estimates of the association under study.

Lastly, Chapter 5 integrated environmental, entomological, animal and human data to model *Leishmania infantum* transmission in the Piedmont region, applying geostatistical and spatial regression models. This study embraces a

three-step modelling strategy: (i) the estimation of the spatial distribution of *P. perniciosus* through a geostatistical model including 4 environmental parameters as predictors, (ii) the estimation of the probability of each municipality of being affected by canine leishmaniasis through a logistic model including the spatial distribution of *P. perniciosus* as a predictor, (iii) the estimation of the relationship between the estimated presence of canine leishmaniasis and the count of human Visceral Leishmaniasis cases through a negative binomial model at the municipality level. From a methodological point of view, several developments are possible. For example, there is the opportunity to propagate the uncertainty from one model to the other through Monte Carlo techniques or, alternatively, to develop a unique multi-stage model analyzing jointly environmental, entomological, animal and human data.

## 6.2 Major findings on WNV

Chapters 2, 3, and 4 focused on WNV in order to elucidate the WNV epidemiology in Italy. The WNV infects birds as primary host and it was likely introduced in Europe by migratory birds. WNV was detected in Italy for the first time in 1998, during an outbreak among horses in Tuscany<sup>80</sup>, and then re-emerged ten years later in the northern areas of the country, causing regular human outbreaks<sup>71</sup>. Several scientific questions about the WNV eco-epidemiology in Italy remain unsolved. While WNV can be now considered endemic in Italy, its persistence mechanism is still unknown. It might survive from one year to the next in the local avian fauna<sup>81</sup>, in overwintering mosquitoes<sup>82</sup> or it might be reintroduced each year with migratory birds<sup>83</sup>. In addition, predicting where and when WNV outbreaks will occur and how intense they will be is challenging, as viral transmission risk varies according to environmental, climatic, entomological and human factors<sup>84</sup>. For instance, in 2018, the WNV transmission season started much earlier compared to previous years, determining a sharp increase in the number of human WNV infections<sup>85</sup>. There is also large uncertainty about the potential impacts of climate change on the WNV ecology. Empirical and modelling studies have suggested that increasing temperatures driven by climate change might favour WNV transmission in several areas of the world, by increasing the length of the transmission season, the vector abundance and the viral replication<sup>39,86–89</sup>.

In Chapter 2 we assessed the spatio-temporal distribution of all WNND cases observed in Italy since 2008, when the first human case was detected, to identify high risk areas for WNV outbreaks. Over the 10 years under study, we observed a constant increase in incidence and a geographical shift from east to west, mainly in the provinces along the Po River, in the North of Italy. Results suggested that the Po River and its neighbouring areas might present environmental, meteorological and ecological conditions that can promote WNV transmission and circulation.

To investigate the role of meteorological parameters in driving the WNV outbreaks, we conducted two studies in Northern Italy, presented in Chapter 3 and 4. In Chapter 3, we found evidence of an association between the maximum temperatures recorded in the 5<sup>th</sup> and 6<sup>th</sup> weeks prior to diagnosis (lags 5 and 6) and the incidence of human WNV infection. We also observed an association between the incidence of WNV infection cases and the total precipitation recorded between 1 and 4 weeks prior to diagnosis. In Chapter 4,

we reported the results from the entomological surveillance conducted during the 2018 in Lombardy and Emilia-Romagna, two of the most affected regions by the WNV circulation. This study includes analyses on the meteorological determinants of the WNV circulation among *Culex* mosquitoes. We observed a positive relationship between the absolute number of collected mosquitoes and the temperature recorded one week before sampling, as well as between the number of WNV-positive mosquitoes and the temperature recorded two weeks before sampling. No evidence of association was detected between the two entomological parameters and the weekly number of wet days observed in the previous 4 weeks.

The latter two studies provided consistent results about the role of air temperatures on the WNV dynamics. Air temperatures appear to be associated with WNV infected mosquitoes with a 2 weeks lag, and with infected WNV human cases with a 5-6 weeks lag. The 2 weeks delay of the temperatures effect on the positive mosquitoes is in line with previous findings and with the time of the virus extrinsic incubation in the *Culex* mosquitoes<sup>90,91</sup>. The extra 3 weeks needed to observe an effect on the occurrence of human cases could be explained by the time of virus transmission from mosquitoes to humans, the incubation period, and the time from symptom onset to the diagnosis<sup>92,93</sup>. These results support the idea that air temperature is one of the major environmental drivers influencing WNV epidemiology.

However, these two studies provided inconsistent results about the role of precipitation on WNV dynamics. No evidence of association was detected between the weekly number of wet days and entomological parameters, while incident cases of WNV infection resulted associated with total precipitation recorded between 1 and 4 weeks prior the diagnosis. These results could be due to the different way the information of precipitation amount was expressed (number of wet days *vs* total amount) and assessed (interpolation *vs* land-based recording). However, the literature about the role of precipitation on the WNV also provides contradictory findings<sup>39</sup>.

## 6.3 Major findings on Leishmaniasis

In Chapter 5 we integrated environmental, entomological, animal and human data to model the *Leishmania infantum* transmission risk in Piedmont, to evaluate its current distribution and whether *L. Infantum* can be now considered endemic in this region.

Leishmaniasis is a vector-borne disease with a broad global occurrence<sup>94</sup>. In Europe, two species of *Leishmania* are the main putative agents for endemic human disease: *L. tropica* (cutaneous leishmaniasis) and *L. infantum* (visceral leishmaniasis)<sup>60</sup>. In Europe, *Leishmania* causes sporadic outbreaks, and the disease is often neglected by public health authorities. The parasites are transmitted to susceptible organisms, such as humans and dogs, by the bite of female phlebotomine sand flies. Given the complex pathogen-vector-host interaction, the geographical distribution of leishmaniasis is influenced by a variety of environmental, landscape, and socioeconomic factors<sup>95</sup>. Over the last decades, the pathogen and its vectors have been found to expand geographically, increasing the probability of local outbreaks<sup>96</sup>. The reported range of expansion of the diseases has been associated with the range of expansion of vector populations in response to climate change<sup>97</sup>. However, evidence for the contribution of climate change on the distribution of human cases of Visceral leishmaniasis is lacking.

It has been suggested that the warming trends observed in the last decades might have acted as a potential driver of the shift of the geographical distribution of Leishmaniasis towards northern Italian regions, such as Piedmont<sup>61</sup>. Interestingly, in the last decades, the Piedmont region experienced increasing temperatures, with the average maximum temperatures increased by 2.3°C. and the average minimum temperatures increased by 1.5 °C in the last 60 years<sup>98</sup>. In chapter 5, we used geostatistical modelling to identify environmental factors (such as altitude, temperature, and NDVI) associated with the presence of *P. perniciosus*, the main vector of *L. infantum* in Italy. In particular, we detected a positive association between *P. perniciosus* and the average daily summer temperatures. In addition, through spatial modelling, we observed that the geographical distribution of the vector, infected reservoir hosts and human cases are associated. Our findings indicate that *L. infantum* strains have been actively circulating in some areas of the region since the beginning of the 21<sup>st</sup> century. Our results can inform local health authorities about high risk areas and help to implement public health intervention.



## 6.4 Future perspectives

Our studies confirm the potentiality of integrating data about human cases, invasive or endemic vector species with environmental, meteorological and climatic data for the modelling of vector-borne diseases. Results provide insights into the mechanisms underlying the circulation of two vector-borne diseases currently endemic in Italy. These results might stimulate future studies aimed at investigating the causal link between environmental and meteorological parameters with the circulation of vector-borne diseases and hypothesize future scenarios driven by climate change.

Concerning WNV, starting from the results provided by the study on WNND spatio-temporal spread (Chapter 2), we would like to test whether warmer winter temperatures can lead to the survival of infected mosquitoes (overwintering phenomenon) and, consequently, to a higher number of WNV cases in the following season. In addition, the observed lag-effects between meteorological and entomological/epidemiological outcomes (Chapter 3 and 4) might inform climate-driven predictive models to forecast, at the beginning of the transmission season, when and where the viral circulation will be higher, both among vectors and humans<sup>99–101</sup>. This information could be used to implement early warning systems able to implement public health interventions promptly. Lastly, identified drivers of WNV circulation might be combined with predictions of future climate-change scenarios to generate projections of the potential future effects of climate change and indicate areas that are likely to become more or less suitable for the transmission of WNV<sup>73</sup> in the next decades.

Concerning Leishmaniasis, our study depicts the epidemiological scenario of *L. infantum* in Piedmont in the first decade of the 21<sup>st</sup> century and might be useful for comparison with future observations. Knowledge of the epidemiological scenario at a specific time point will help to understand whether and how the geographical distribution of *L. infantum* and abundance of its vector are changing in the next years, and to elucidate whether altered climatic conditions have acted as drivers of such changes. In addition, identification of suitable areas for the *L. infantum* can be used to set up surveillance systems among humans, dogs and sand flies as well as to address public health interventions in high risk areas.

## CHAPTER 7

### Conclusions

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This research project attempted to assess the potential effects of environmental, meteorological and climatic parameters on the spread of two vector-borne diseases in Italy, with a focus on the potential impact of climate change. It has been suggested that climate will change and shape the risk profiles of different diseases throughout the world<sup>102,103</sup>. Italy is located in the middle of the Mediterranean basin and it is particularly exposed to the negative effects of a changing climate. The increasing mean temperature recorded in the last decades and the increasing frequency of extreme weather events, as heatwaves, had a non-negligible effect on the population health. Italy has experienced one of the highest heat-related mortality risks among European countries<sup>16</sup>. Increased risk of heat-related adverse health outcomes is greater in large urban areas (Turin, Milan, Bologna, Florence, Rome, Naples), where temperatures are usually higher than in the surroundings rural areas (urban heat island). Urban areas are also characterized by poorer air quality levels that can worsen the health effects of heatwaves. Italy is also experiencing more and more other extreme weather events such as local storms, river flooding, wildfires, landslides. Although single extreme events cannot be directly attributed to anthropogenic climate change, its role in changing the likelihood of occurrence and the intensity of these events is recognized<sup>104</sup>. A recent research has underlined that Italy is one of the Western countries that are experiencing the highest number of deaths attributable to extreme weather events<sup>105</sup>. In addition, hot temperatures and drought can lead to water scarcity and to a reduction in agricultural production and desertification.

Changes in the risk of vector-borne diseases is another potential mechanism through which global warming can affect human health. While globalization could favor the introduction of exotic pathogens and vectors<sup>45</sup>, climate change can determine favorable meteorological and climatic conditions for the survival of the newly established pathogens and vectors and trigger the spread of endemic pathogens and vectors into new areas<sup>42,46</sup>. Italy, in the last decades, has been observing a drastic change in the presence of invasive species of competent vectors and pathogens. For instance, it has become endemic for different species of *Aedes* mosquitoes (*A. albopictus*, *A. koreicus*, *A. japonicus*),

that are potential vectors of important arboviruses<sup>48,106,107</sup>. Outbreaks of Chikungunya were reported in Italy in 2007 and 2017<sup>108,109</sup>, while West Nile Virus has been actively circulating among northern Italian regions since 2008<sup>71</sup>. Concurrently, the geographical distribution of endemic pathogens, as *L. infantum*, is changing in Italy with a northward shift. In conclusion, our results provide insights into the mechanisms underlying the circulation of two climate sensitive diseases currently endemic in Italy and might lay the foundations for future studies as well as help the planning of future public health interventions.

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## APPENDIX

### Climate Change and the COVID-19 Pandemic

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This research project aimed to explore the role of environmental, meteorological and climatic parameters on the epidemiology of vector borne diseases in Italy, paying specific attention to the potential effects of climate change. However, given the unexpected COVID-19 emergency, I spent the last six months of my PhD addressing scientific questions regarding the COVID-19 pandemic. In this appendix, I will describe the research activities I conducted in the last months, preceded by a short introduction aimed at comparing the two global health crises: the COVID-19 Pandemic and the Climate Change.

As discussed at the beginning of this thesis, climate change can be considered a health-crisis. Climate change is already massively affecting human health through different mechanisms, as direct effects of extreme weather events, increase activity of infectious disease, disruption of ecological systems and food yields, human conflicts and migration. With millions of deaths attributable to air pollution and climate change each year, fossil fuels represent one of the biggest health threats of the 21st century. To limit disruptive effects of climate change both on the natural and human system, scientists warned to adopt massive efforts in order to limit global warming to 1.5°C above pre-industrial levels, by cutting greenhouse gas emissions over the next decades with the aim of reaching net zero GHG emissions globally around 2050<sup>4</sup>.

In late 2019, Coronavirus disease 19 (COVID-19) caused an unprecedented health and economic global crisis, summing up to the climate one. As July 15<sup>th</sup>, 2020, nearly 13.6 million people had been infected with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) globally, and more than 500,000 deaths had been reported<sup>1</sup>. Even though apparently dissimilar, the Covid-19 and climate crisis have many common

features as they both affect several aspects of our society (e.g. health and economy), represent serious cross-border threats and will probably impact the most vulnerable societies and the most vulnerable groups within each society. The two crises, however, are possibly different in their intensity and length, the first being sharp and probably limited in time, the second being gradual and prolonged.

The two crises are bounded also by another aspect: COVID-19 pandemic has led to a drastic decrease in energy consumption and GHG emissions, as many governments adopted control measures to limit the transmission of the virus, by imposing quarantine to their citizens, reducing transport and productive activities. The GHG emission decrease in 2020 annual has been recently estimated to be between –2% and –13%, depending on the length and the nature of restrictions<sup>2</sup>. Projected GHG reduction will probably be the biggest reduction (both in absolute and relative terms) ever observed, however happening at the expenses of a severe health, social and economic impacts whose consequences we can just start figuring out. Interestingly, the projected yearly reduction in GHG emissions caused by the disruptive COVID-19 crisis is in line with the reduction suggested by the Intergovernmental Panel on Climate Change (IPCC) optimistic scenario that contemplates a 7% yearly reduction of GHG emission to reach the net zero in the next 30 years. Unfortunately, the decrease of GHG emissions in 2020 is likely to be temporary as it does not reflect structural changes in the industrial, transport or energy systems. A fundamental lesson we must take from the COVID-19 crisis is that the roadmap to a society free from fossil fuel is steep, but imperative, and we must think new structural sustainable scenarios for the post-pandemic phase able to conjugate human wellbeing and GHG reduction in order to mitigate a second prolonged health crisis driven by climate change that will follow the abrupt Covid-19 crisis.

1. COVID-19 Map - Johns Hopkins Coronavirus Resource Center. Available at: <https://coronavirus.jhu.edu/map.html>. (Accessed: 22nd July 2020)
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## List of Published Manuscript

### A) Original article:

#### **Approaches to Daily Monitoring of the SARS-CoV-2 Outbreak in Northern Italy.**

**Giovenale Moirano**, Lorenzo Richiardi, Carlo Novara and Milena Maule.

*Front Public Health.* 2020; **8**: 222

**Abstract:** Italy was the first European country affected by the Sars-Cov-2 pandemic, with the first autochthonous case identified on Feb 21st. Specific control measures restricting social contacts were introduced by the Italian government starting from the beginning of March. In the current study we analyzed public data from the four most affected Italian regions. We (i) estimated the time-varying reproduction number ( $R_t$ ), the average number of secondary cases that each infected individual would infect at time  $t$ , to monitor the positive impact of restriction measures; (ii) applied the generalized logistic and the modified Richards models to describe the epidemic pattern and obtain short-term forecasts. We observed a monotonic decrease of  $R_t$  over time in all regions, and the peak of incident cases  $\sim 2$  weeks after the implementation of the first strict containment measures. Our results show that phenomenological approaches may be useful to monitor the epidemic growth in its initial phases and suggest that costly and disruptive public health controls might have had a positive impact in limiting the Sars-Cov-2 spread in Northern Italy.

**A) Original article:**

**Higher testing coverage is associated with lower COVID-19 mortality rate: insights from Italian regions.**

Andrea Saglietto\*, **Giovenale Moirano\***, Matteo Anselmino, Gaetano Maria De Ferrari.

\* These authors equally contributed to the manuscript

*Disaster Med Public Health Prep, 2020;1-11*

**Abstract:** Different COVID-19 testing approaches have been implemented among Italian regions, reflected in the heterogeneous number of swabs testing rates performed. We analysed the number COVID-19 related deaths in relation to the number of swabs tests performed among the most hit Italian regions. We observed that regions with the highest number of tests performed (Veneto and Toscana) had the lowest 30-day cumulative mortality rate (per 100,000 inhabitants). In addition, we observed an inverse association between cumulative mortality rates and tests performed (Incidence Mortality Rate Ratio (IMRR) for unit increase in swabs tests per 1,000 inhabitants: 0.92; 95% CI 0.890-0.94). Our short report suggests that early identification and isolation of active cases (including asymptomatic or mildly symptomatic subjects) could have had an important effect in lowering COVID-19 mortality.

**B) Letter to the Editor:**

**Short-Term Effects of Mitigation Measures for the Containment of the COVID-19 Outbreak: An Experience from Northern Italy.**

**Giovenale Moirano**, Maurizio Schmid, Francesco Barone-Adesi.

*Disaster Med Public Health Prep, 2020;1-2.*

**Abstract:** We evaluated the short-term effects of mitigation measures imposed by the Italian government on the first 10 municipalities affected by Sars-Cov-2 spread. Our results suggest that the effects of containment measures can be appreciated in about approximately 2 wk.

## List of Submitted Manuscript

### A) Original Article:

#### **Does death from Covid-19 arise from a multistep process?**

Neil Pearce, **Giovenale Moirano**, Milena Maule, Manolis Kogevinas, Xavier Rodo, Deborah Lawlor, Jan Vandenbroucke, Christina Vandenbroucke-Grauls, Fernando P Polack, Adnan Custovic.

Submitted to *European Journal of Epidemiology*

**Abstract:** Covid-19 death has a different relationship with age than is the case for other severe respiratory pathogens. The Covid-19 death rate increases exponentially with age, and the main risk factors are age itself, as well as having underlying conditions such as hypertension, diabetes, cardiovascular disease, severe chronic respiratory disease and cancer. Furthermore, the almost complete lack of deaths in children suggests that infection alone is not sufficient to cause death; rather, one must have gone through a number of changes, either as a result of undefined aspects of aging, or as a result of chronic disease. These characteristics of Covid-19 death are consistent with the multistep model of disease, a model which has primarily been used for cancer, and more recently for amyotrophic lateral sclerosis (ALS). We applied the multi-step model to data on Covid-19 case fatality rates (CFRs) from China, South Korea, Italy, Spain and Japan. In all countries we found that a plot of  $\ln(\text{CFR})$  against  $\ln(\text{age})$  was approximately linear with a slope of about 5. As a comparison, we also conducted similar analyses for selected other respiratory diseases. SARS showed a similar log-log age-pattern to that of Covid-19, albeit with a lower slope, whereas seasonal and pandemic influenza showed quite different age-patterns. Thus, death from Covid-19 and SARS appears to follow a distinct age-pattern, consistent with a multistep model of disease that in the case of Covid-19 is probably defined by comorbidities and age producing immune-related susceptibility. Identification of these steps would be potentially important for prevention and therapy for SARS-COV-2 infection.

**B) Original article:**

**Impacts of Reopening Strategies for COVID-19 Epidemic: A Modelling Study in Piedmont Region.**

Simone Pernice; Paolo Castagno; Linda Marcotulli; Milena Maria Maule; Lorenzo Richiardi, **Giovenale Moirano**; Matteo Sereno; Francesca Cordero; Marco Beccuti.

Submitted to *BMC Infectious Diseases*

**Abstract:** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the coronavirus disease 19 (COVID-19), is a highly transmittable virus. Since the first person-to-person transmission of SARS-CoV-2 was reported in Italy on February 21-st, 2020, the number of people infected with SARS-CoV-2 increased rapidly, mainly in northern Italian regions, including Piedmont. A strict lockdown was imposed on March 21-st until May 4-th when a gradual relaxation of the restrictions started. In this context, computational models and computer simulations are one of the available research tools that epidemiologists can exploit to understand the spread of the diseases and to evaluate social measures to counteract, mitigate or delay the spread of the epidemic. This study presents an extended version of the Susceptible-Exposed-Infected-Removed-Susceptible (SEIRS) model accounting for population age structure. The infectious population is divided into three sub-groups: undetected infected individuals, quarantined infected individuals and hospitalized infected individuals. Moreover, the strength of the government restriction measures and the related population response to these are explicitly represented in the model. The proposed model allows us to investigate different scenarios of the COVID-19 spread in Piedmont and the implementation of different infection-control measures and testing approaches. The results show that the implemented control measures have proven effective in containing the epidemic, mitigating the dangerous impact of a large proportion of undetected cases. We also forecast the optimal combination of individual-level measures and community surveillance to contain the new wave of COVID-19 spread after the re-opening work and social activities. Our model is an effective tool useful to investigate different scenarios and to inform policy makers about the potential impact of different control strategies. This will be crucial in the upcoming months, when very critical decisions about easing control measures will need to be taken.



**C) Original article:**

**COVID-19-like symptoms and their relation to SARS-CoV-2 epidemic in children and adults of the NINFEA birth cohort.**

Maja Popovic, Chiara Moccia, Elena Isaevska, **Giovenale Moirano**, Costanza Pizzi, Daniela Zugna, Franca Rusconi, Franco Merletti, Milena Maule, Lorenzo Richiardi.

Submitted to *Scientific Report*

**Abstract:** Emerging COVID-19 pandemic caused extensive lockdowns in a number of countries, but yet unknown number of cases positive to SARS-CoV-2 escapes surveillance systems. Mothers participating in the Italian NINFEA birth cohort were invited to complete an online questionnaire on COVID-19-like symptoms in the household. We estimated the population prevalence of COVID-19-like symptoms in children and adults, assessed their geographical correlation with the cumulative number of COVID-19 cases by province, analysed their clustering within families, and estimated their sensitivity, positive (PPV) and negative predictive values (NPV) for COVID-19 diagnosis in individuals tested for SARS-CoV-2. Information was collected on 3184 households, 6133 adults, and 5751 children. There was a strong geographical correlation between the population cumulative incidence of COVID-19 and the prevalence of muscle pain, fatigue, low-grade fever, and breathing difficulties in adults (Spearman's  $\rho \geq 0.70$ ). Having at least one family member with a COVID-19 diagnosis, compared with none tested for SARS-CoV-2, was associated with an increased prevalence ratio of almost all COVID-19-like symptoms in adults, and only of low-grade fever (37-37.5°C; prevalence ratio 5.27; 95% confidence intervals: 2.37 to 11.74) and anosmia/dysgeusia in children. Among adults with COVID-19, fatigue, muscle pain, and fever had a sensitivity  $\geq 70\%$ . In individuals tested for SARS-CoV-2, with a 16.6% prevalence of COVID-19, breathing difficulties and nausea/vomiting had the highest PPVs, with point estimates close to 60%, and with NPVs close to 90%. Among tested Piedmont residents, with a COVID-19 prevalence of 18.5%, breathing difficulties and anosmia/dysgeusia reached PPVs above 80%. Geographical prevalence of COVID-19-like symptoms in adults may inform on local disease clusters, while certain symptoms in family members of confirmed COVID-19 cases could help identification of the intra-familial spread of the virus and its further propagation in the community. Low-grade fever is frequent in children with at least one household member with COVID-19 and possibly indicates child infection.

#### D) Original article:

##### **Factors associated with self-perceived knowledge of COVID-19: a study among women from the NINFEA birth cohort.**

Chiara Moccia, Maja Popovic, Elena Isaevska, **Giovenale Moirano**, Costanza Pizzi, Franca Rusconi, Franco Merletti, Milena Maule, Lorenzo Richiardi.

Submitted to *Epidemiologia e Prevenzione*

**Abstract:** The worldwide spread of COVID-19 led to the need of strategies to contain and control the spread of the virus. Health literacy can be an important too, since individuals need to acquire promptly new health information, understand the reasons behind recommendations, and adapt their behavior accordingly. We investigated socio-demographic-, disease- and pandemic-related factors that can influence self-perceived knowledge (poor/medium vs. high) about COVID-19 in women of the Italian NINFEA birth cohort. In April 2020, a cross-sectional web-based survey on COVID-19 was administered to women participating in the NINFEA cohort. Using multiple weighted logistic regression models, we analyzed self-perceived knowledge level in relation with the following determinants: age, education level, region and area of residence, family size and cumulative incidence of COVID-19 positive cases until April 7th by province. We also assessed if the presence of COVID-19-like symptoms, SARS-Cov-2 testing and COVID-19 diagnosis were associated with the self-perceived knowledge. 3129 women were included in the study. The weighted prevalence of self-perceived poor/medium knowledge was 57%. In multivariable logistic regression analyses, the odds ratio(OR) of self-perceived poor/medium COVID-19 knowledge level was elevated for low education compared with high education level (OR: 1.57; 95% confidence intervals (CI):1.34;1.84), and decreased for SARS-CoV-2 testing (OR 0.25; 95% CI: 0.16;0.39) and COVID-19 diagnosis (OR 0.20; 95% CI: 0.07;0.60). There was no evidence of association between the other analyzed variables and self-perceived level of knowledge. Women education level, having experienced the disease and having been tested for SARS-CoV-2 are the strongest determinants of self-perceived knowledge on COVID-19. Health education programs aimed at improving COVID-19 specific knowledge and confidence in self-perceived knowledge could help the implementation of appropriate practices.



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