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

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Short Title: **Spatio-temporal distribution of West Nile Neuro-invasive Disease in Italy**

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Summary

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 percent 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.

Key words:

West Nile Virus, West Nile Neuroinvasive Disease, Spatio-temporal pattern

Bullet points:

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

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 viremia 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 viremia 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 of 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 Volvograd, 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, Jimenez-Clavero, 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, Jimenez-Clavero, 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 (Rizzo et al., 2012, Napoli et al, 2013, Rizzo et al., 2016). 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.

Methods

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 in 20 regions and in 110 provinces. As the Italian Peninsula 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.

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 (Rizzo et al., 2009; Rizzo et al. 2012, ISS, 2018). 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).

Statistical analysis and results representation

We first analysed the annual incidence rates of WNND cases notified in Italy between 2008-2017. 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 et al., 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 longitude of the capital of the province in which the diagnosis of the case was posed.

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

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 Volvograd lineage 2, was detected in 2014 (Ravagnan et al., 2015), suggesting that 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 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 wet lands and irrigated croplands, highly fragmented natural sites, local bird abundance and high density of Culex mosquitos (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 mosquitos (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 of 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 define as endemic areas provinces in which 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 an active surveillance based on the research of WNV antibodies among both hospitalised 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 accordingly 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 of WNV incidence, useful to predict areas and periods at risk of WNV outbreaks.

Conflict of interest: nothing to declare

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Table1: 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

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 Percent Change (%)

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

Legend Figure 3

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.

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.

Legend Figure 4

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