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

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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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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 (DNLM), 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.