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West Nile virus 'circulation' in Vojvodina, Serbia: Mosquito, bird, horse and human surveillance

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ABSTRACT

Efforts to detect West Nile virus (WNV) in the Vojvodina province, northern Serbia, commenced with human and mosquito surveillance in 2005, followed by horse (2009) and wild bird (2012) surveillance. The knowledge obtained regarding WNV circulation, combined with the need for timely detection of virus activity and risk assessment resulted in the implementation of a national surveillance programme integrating mosquito, horse and bird surveillance in 2014. From 2013, the system showed highly satisfactory results in terms of area specificity (the capacity to indicate the spatial distribution of the risk for human cases of West Nile neuroinvasive disease - WNND) and sensitivity to detect virus circulation even at the enzootic level. A small number ($n = 50$) of *Culex pipiens* (*pipiens* and *molestus* biotypes, and their hybrids) females analysed per trap/night, combined with a high number of specimens in the sample, provided variable results in the early detection capacity at different administrative levels (NUTS2 *versus* NUTS3). The clustering of infected mosquitoes, horses, birds and human cases of WNND in 2014–2015 was highly significant, following the south-west to north-east direction in Vojvodina (NUTS2 administrative level). Human WNND cases grouped closest with infected mosquitoes in 2014, and with wild birds/mosquitoes in 2015. In 2014, sentinel horses showed better spatial correspondence with human WNND cases than sentinel chickens. Strong correlations were observed between the vector index values and the incidence of human WNND cases recorded at the NUTS2 and NUTS3 levels. From 2010, West Nile virus was detected in mosquitoes sampled at 43 different trap stations across Vojvodina. At 14 stations (32.56%), WNV was detected in two different (consecutive or alternate) years, at 2 stations in 3 different years, and in 1 station during 5 different years. Based on these results, integrated surveillance will be progressively improved to allow evidence-based adoption of preventive public health and mosquito control measures.

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1. Introduction

West Nile virus (WNV), family *Flaviviridae*, is the most widespread arbovirus in the world. It started to cause growing concerns in Europe after the largest outbreak in Romania in 1996, which was connected to many cases of neuroinvasive disease (WNND) in humans [1,2].

The virus may disappear or remain undetected for long periods, but different environmental "drivers", such as an increase in temperature, may enhance virus circulation and affect humans and equids [3]. In Europe, the main vector is *Culex pipiens* (including the *pipiens* and *molestus* biotypes, and their hybrids) [1,4,5], but *Cx. perexigus* and *Cx. modestus* may act as a bridge between birds and humans/horses in specific areas [6]. The virus may overwinter in infected female mosquitoes, as well as in residential birds, and could circulate locally without re-introduction via migrating birds [7–9].

The largest WNV outbreak in Europe, with more than 390 confirmed cases, was reported in Romania in 1996 [1]. Serbia experienced the second largest outbreak, with 200 confirmed human cases in 2013 [10]. The largest numbers of human cases, as well as outbreaks of various magnitudes, have been reported in the Vojvodina province of northern Serbia since 2012 (9 in 2012, 85 in 2013, 27 in 2014 and 10 in 2015) [11]. According to the nomenclature of territorial units for statistics (NUTS), Vojvodina corresponds to the NUTS2 administrative level [12].

Little is known about the history of WNV circulation in Serbia. The first serological investigation was conducted in 1972, and antibodies against WNV were found in 2.6%–4.7% of human serum samples [13]. After more than a 30-year gap, the authors of this paper resumed serological investigation, first of human sera. ELISA IgG testing revealed that the seroprevalence of WNV was 6.67% in human sera from 45 patients who had been hospitalized for encephalitis or meningoencephalitis between 2001 and 2005. The seroprevalence was 3.69% in 406 samples taken from healthy people. The average seroprevalence of WNV in samples taken from 2001 to 2009 was 3.99% (18 of 451). A total of 337 individuals tested in 2009 were exposed to at least one mosquito exposure-related risk factor. Within this group, 5.04% were seropositive for WNV. Most of the probably infected people (those with IgG in blood sera) did not have screens on the windows and doors of their houses, while only 0.88% of those using window screens were seropositive for WNV [5]. During the same period, 56,757 mosquito specimens, sampled at migratory and domestic bird reservoirs, were pooled into 841 samples and all tested negative for WNV RNA. A serological analysis by ELISA based on WNV recombinant envelope E (rE) protein and PRNT showed, for the first time in Serbia, that 12% of 349 horses from the northern part of country sampled from 2009 to 2010 presented with specific neutralizing WNV antibodies [14]. Due to the absence of routine diagnosis and the limited resources of hospitals in Serbia, human cases of meningoencephalitis of unknown origin that should have been evaluated by a plaque reduction neutralization test and/or RT-PCR tests for WNV were not tested until 2012. In addition, regular sentinel chicken, horse or mosquito surveillance did not exist. Consequently, the approach used to search for the virus in Serbia had been focused on IgG-positive humans to find infected mosquitoes, i.e., to provide evidence of circulation and raise the public awareness of the risk. In accord with the negative results, the lack of the resources and resultant planning, the sampling of mosquitoes in 2010 and 2011 was performed at/around places where humans or equids that were IgG-positive or infected with WNV were recorded in the previous season. The plan implemented had generated initial results in 2010 when WNV linage 2 RNA was detected in three pools of *Cx. pipiens* [5]. In August 2012, a clinical outbreak of WNV infection in humans was reported for the first time in Serbia [15,16]. In addition, during the same year, viral RNA was detected for the first time in nine wild birds. All of the isolates were classified by phylogenetic analysis as lineage 2 WNV strains and were closely related to the strains responsible for recent outbreaks in Greece, Italy and Hungary [17].

The prevention and control of WNV outbreaks is complex and requires the implementation of a comprehensive surveillance system [18,19]. Environmental surveillance based on mosquito and/or bird collections and subsequent screening for the pathogen has been shown to perform well in detecting the virus circulation well before the occurrence of human cases, and also enables estimations of the magnitude of human WNV outbreaks, with a possibility for the identification of affected areas [18,20]. During the first period, from 2005 to 2013, WNV surveillance activities in Vojvodina were performed as part of ongoing research projects coordinated by the authors of this paper. In 2014, a specific and integrated surveillance system targeting mosquitoes, wild and sentinel birds as well as horses, was set up by the National Veterinary Directorate in Vojvodina province, northern Serbia. The main goals of this nationwide WNV surveillance have been to provide warnings of WNV circulation and evidence-based tools for controlling the spread of WNV infections in humans.

In this paper, the development of the surveillance system in the five years following the first detection (2010) of WNV RNA in *Cx. pipiens* mosquitoes in Serbia is presented. We also present a comparison between the results obtained from mosquito, bird, horse and human populations from 2014 to 2015 and describe their spatial clustering in Vojvodina province. In addition, the incidence of human WNND cases was correlated to the vector index values for *Cx. pipiens* for different sizes of territorial/administrative levels.

2. Material and methods

Vojvodina has a total surface area of 21,506 km^2 with a population of 1.93 million. The climatic and ecological conditions for *Cx. pipiens* (including biotypes *pipiens* and *molestus*, and their hybrids) development (e.g., availability of breeding sites, bird species populations and environmental parameters) are very similar all around the province and are considered appropriate for WNV circulation.

2.1. Surveillance of mosquitoes

Following the detection of the WNV infection in mosquitoes in the Vojvodina province in 2010 [5], a small-scale surveillance network was designed and operated during the late summer period (August–September) (Table 1). In the 2011-13 seasons, mosquito collections were conducted at places where humans or equids that were IgG-positive or infected with WNV had been recorded in the previous season. Surveillance in 2013 covered all of the districts of Vojvodina with human WNND cases. Traps baited with dry ice and without light (NS2 type) were operated from the afternoon until the morning of the next day (one trap night), some were set at fixed positions and others were irregularly placed, with weekly to biweekly periodicity.

From the 2014 season, the mosquito collections were standardized and traps were fixed to geo-referenced positions with biweekly periodicity (June–September) within high-risk (estimated according to number of human cases in the previous season) districts (NUTS3 level) of the province (NUTS2 level). In 2014, all districts except the North Banat district were considered to be high-risk. North Banat was sampled at a three-weekly or monthly periodicity (five times between June–September). In 2015, biweekly sampling was performed in all districts. The network was initially designed to cover the area of all districts and as many