

WEST NILE VIRUS OUTBREAK IN CROATIA, 2018

Irena Tabain¹, Tatjana Vilibic-Cavlek¹, Vladimir Savic¹, Ljubo Barbic¹, Dario Sabadi¹, Ljiljana Peric¹, Bozana Miklausic¹, Marija Santini¹, Maja Bogdanic¹, Marko Vucelja¹, Elizabeta Dvorski¹, Tamara Butigan¹, Tanja Potocnik-Hunjadi¹, Gordana Kolaric-Sviben², Ana Klobucar¹, Marina Balicevic¹, Vladimir Stevanovic¹, Krunoslav Capak¹, Eddy Listes², Giovanni Savini³

Arbovirus study group; ¹Collaborators on the project, Croatian Science Foundation: IP-2016-06-7456: "Prevalence and molecular epidemiology of emerging and re-emerging neuroinvasive arboviral infections in Croatia" (CRONEUROARBO); ²Collaborators of the Reference Center for Diagnosis and Surveillance of Viral Zoonoses Croatian Ministry of Health; Croatian Institute of Public Health, Zagreb, Croatia; ³OIE Reference Centre for West Nile Disease, Istituto Zooprofilattico Sperimentale "G. Caporale", Teramo, Italy

INTRODUCTION: West Nile virus (WNV) is one of the most widely distributed arboviruses. In Croatia, first clinical cases of WNV neuroinvasive disease were reported in 2012, thereafter cases were continuously detected in the following transmission seasons. In addition, acute infections as well as IgG seropositivity in horses and poultry were also documented. In 2018, the largest outbreak of WNV occurred with more than 60 human cases. For the first time in Croatia, WNV infection was detected in wild birds. The aims of this study were to analyze epidemiological characteristics and molecular epidemiology of WNV infections detected during 2018 transmission season.

METHODS: From January to December 2018, a total of 182 patients with neuroinvasive disease and 70 patients with symptoms compatible with WNV fever were tested for the presence of WNV RNA, IgM/IgG antibodies and IgG avidity. In addition, a total of 2759 horses were tested for the presence of WNV IgG antibodies. IgG positive serum samples were further tested for IgM antibodies for confirmation of recent infection. Thirty-five dead wild birds were tested for WNV RNA. WNV RNA was detected using a real-time RT-PCR according to the protocol of Tang et al. (2007). WNV RNA positive samples were further tested using a nested RT-PCR and Sanger sequencing. Serological tests were performed using a commercial indirect ELISA (Euroimmun, Lübeck, Germany) or virus neutralization test (human samples) and competitive ELISA (Ingezim West Nile COMPAC, Madrid, Spain; horse samples). Meteorological data (air temperature, precipitation) obtained from the Croatian Meteorological and Hydrological Service were also analyzed.

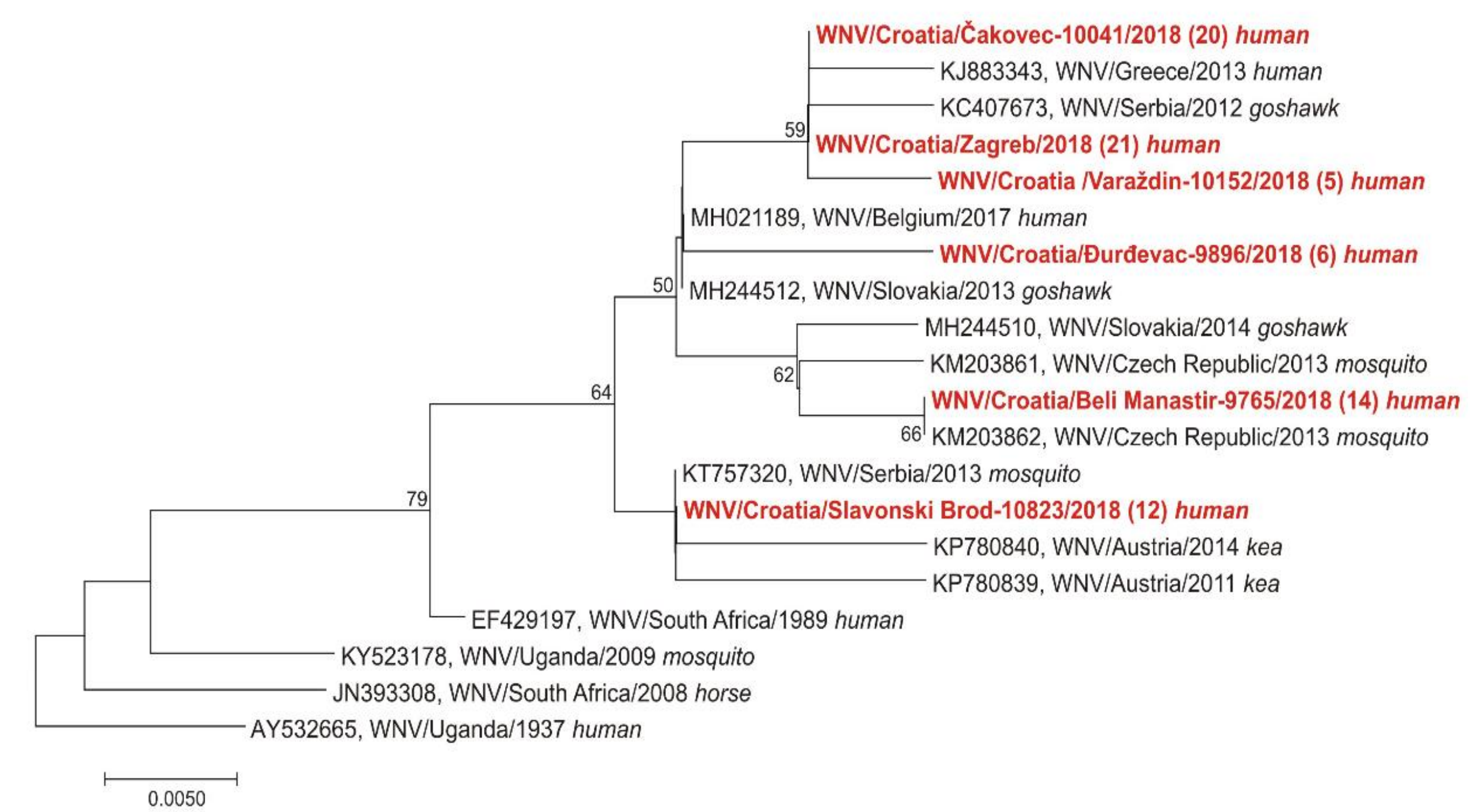


Figure 2. Phylogenetic analysis of human West Nile infections, 2018

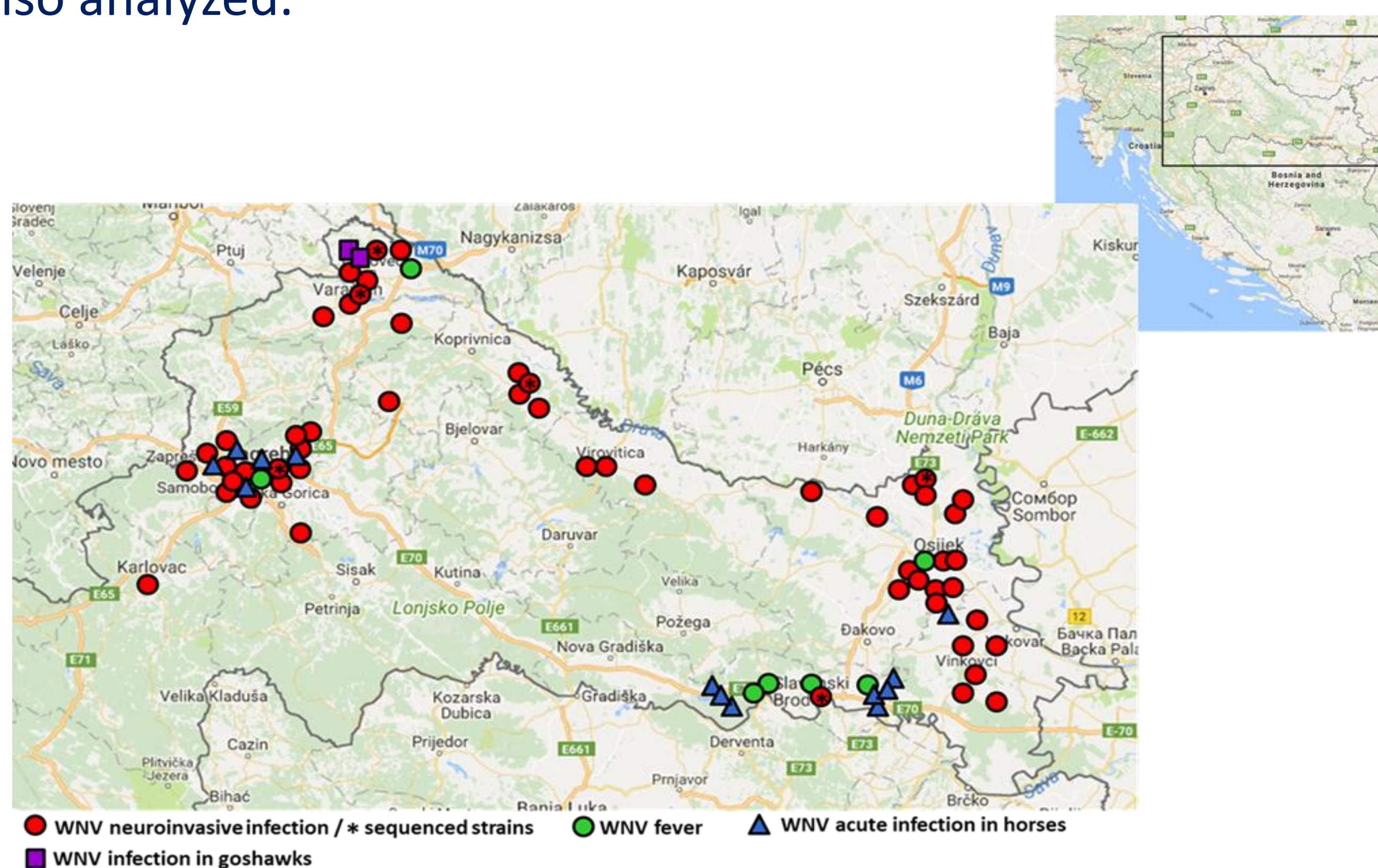


Figure 1. Geographical distribution of West Nile infections detected in 2018 Croatian outbreak

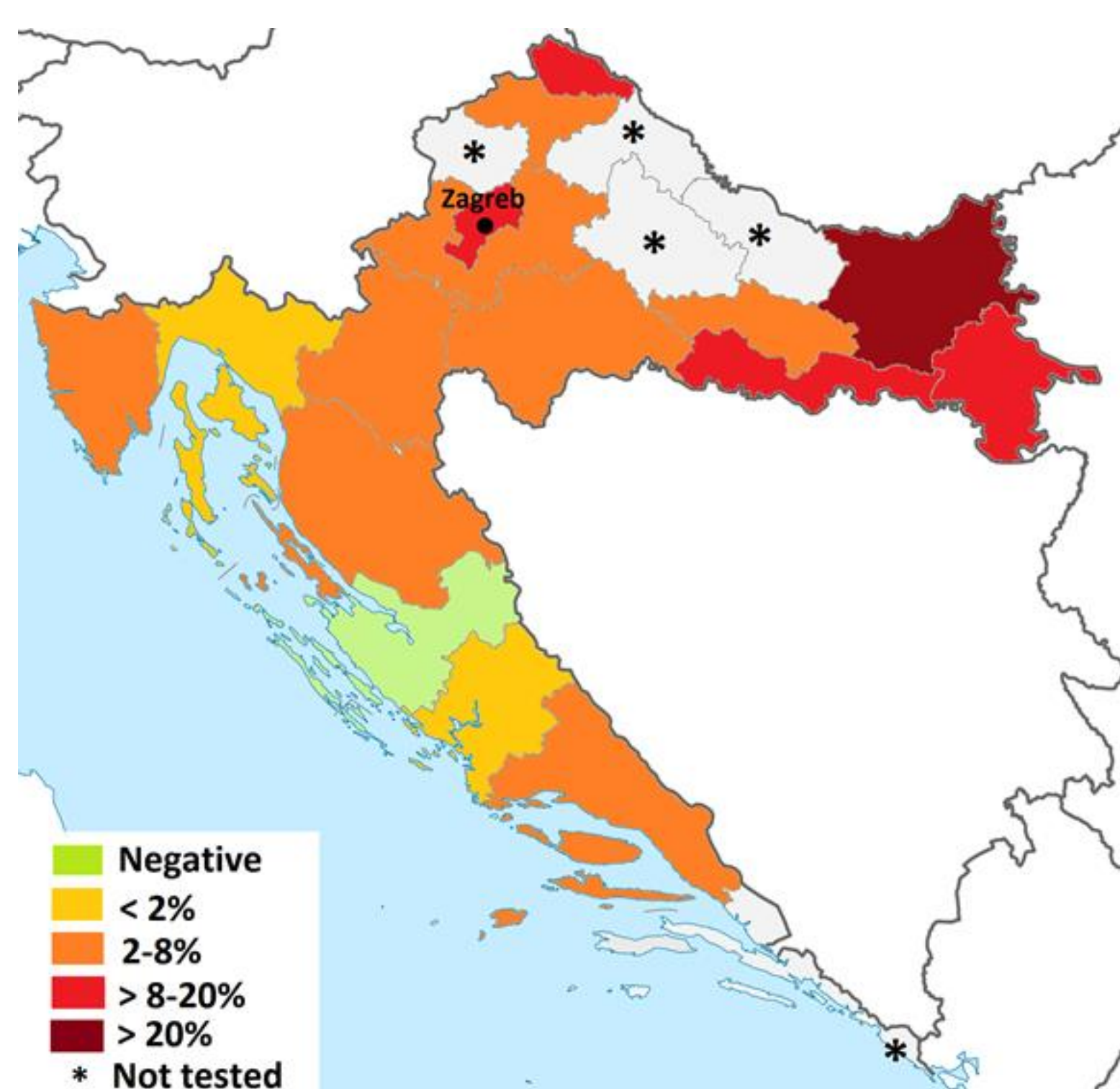


Figure 3. Seroprevalence of West Nile virus in horses, 2018

RESULTS: WNV infection (WNV neuroinvasive disease/WNV fever) was confirmed in 61 patients. Cases occurred in ten continental Croatian counties (figure 1). Phylogenetic analysis of six detected strains showed circulation of WNV lineage 2 (figure 2). Acute asymptomatic infection was documented in 13/0.03% horses from three continental counties while IgG antibodies were detected in 232/8.4% horses. Seroprevalence rates were higher in continental counties (3.2-26.0%) compared to counties on the Adriatic coast (0-4.8%) (figure 3). IgM positive horses were detected from March to June, while human infections occurred from July to October. Two WNV positive goshawks were detected in September. The early start of WNV season was associated with favorable climate conditions (according to percentile ranks and classification ratings, thermal conditions for both months fall under the extremely warm category; 99 percentile).

CONCLUSION: Our results confirm the need of continuous multidisciplinary ("One health") surveillance of this emerging viral zoonosis.



The study was supported by the project, Croatian Science Foundation: IP-2016-06-7456: Prevalence and molecular epidemiology of emerging and re-emerging neuroinvasive arboviral infections in Croatia; CRONEUROARBO (to TVC). Authors thank Ljiljana Milasincic, Ljiljana Antolasic and Snjezana Artl for technical assistance.