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WEST NILE VIRUS SEROSURVEILLANCE IN HORSES IN CROATIA DURING THE 2012 TRANSMISSION SEASON

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Summary

In 2012 the active WNV surveillance system in horses was introduced in Croatia. Between June and October 2012, in six Croatian counties, 1804 horse sera were sampled and tested for IgM WNV antibodies as the confirmation of an acute infection. Additional 1472 samples were tested for the presence of IgG WNV antibodies in the seroprevalence study. The study showed the increased seroprevalence of WNV infection in horses and acute infections in horses in three Eastern Croatian counties. In the same transmission season, the first human WNV clinical cases were reported in the counties with a confirmed increase in WNV seroprevalence in horses. Even more, the first detection of horses acutely infected with WNV had been two weeks before the first confirmed human clinical case. The results confirmed the active serosurveillance system in horses as a valuable tool for WNV surveillance in Croatia and as the source of important veterinary medicine and public health data.

Keywords: West Nile virus; serosurveillance; horses; public health; Croatia.

INTRODUCTION

West Nile virus (WNV) is a zoonotic mosquito-transmitted arbovirus belonging to the genus *Flavivirus* in the family *Flaviviridae*. The first isolation of West Nile virus was documented in Omogo in the West Nile District of Uganda from an adult woman [1]. Subsequently, the virus has been described to be widely distributed in many parts the Old World, such as Africa, the Middle East, Asia, Europe, and elsewhere [2]. During the summer of 1999, WNV was first detected in the Western

Hemisphere in the north-eastern United States of America [3]. For the last two decades the West Nile virus (WNV) infection associated disease outbreaks are occurring worldwide, making it the pathogen of major global public health concern. In Europe in the last few transmission seasons an increased number of WNV infections has been noted in humans and animals and a concern has been raised regarding public and animal health.

In WNV natural cycle birds normally act as amplifying hosts whereas mosquitoes, mainly of the genera *Culex*, *Aedes* and *Ochlerotatus*, play the vector role. In this cycle humans, horses and other mammals are regarded as incidental or dead-end hosts. The members of the order *Passeriformes* (jays, blackbirds, finches, sparrows, crows) and *Columbiformes* (collared doves) seem to be important in maintaining the virus in nature because of their high viraemias and, in opposite, because of their presumed low and transient viraemias, humans and horses are not considered important in the natural transmission cycle [4].

Several WNV lineages have been described so far [5]. In Europe, the lineage 1 is widespread and further segregates into different subclades. Two main routes of lineage 1 dispersion have been identified in Europe, one in Eastern and the second one in Western Europe [6]. Lineage 2 is mainly present in sub-Saharan Africa and Madagascar, but in the last decade was also identified in the Eastern Europe, first time detected in Hungary in 2004 [7]. Since then, lineage 2 has been detected in Rumania [8], Greece [9], Italy [10] Russia [11] and Austria [12]. Along with lineages 1 and 2, WNV lineage 3 has been isolated in the Rabensburg region of the Czech Republic [13] and lineage 4 has been identified in the Southern Russia [14]. WNV strains differ considerably in virulence and neuroinvasiveness. Since neuroinvasive isolates mainly belong to lineage 1, lineage 2 strains are considered to be less virulent. Recent data, however, indicate that the several highly virulent and neuroinvasive strains of lineage 2 WNV have been detected in Southern Africa [15].

The fact that West Nile virus is widespread in many European countries and outbreaks in humans with fatal end cases as the evidence of virus endemisation [16] highlighted the necessity of global surveillance system introduction.

Different WNV surveillance systems have been established in different countries in accordance with the epidemiological situations. In general, WNV control systems include surveillance in human and animal populations and entomological surveillance. All surveillance systems can be performed as passive and/or active systems.

The aim of the human passive surveillance system is the detection of infection in humans and the estimation of its diffusion through the systematic analysis of newly emerging clinical cases. The active human surveillance includes the serolo-

gical testing of people who live or work in the areas of documented WNV circulation. In general, the results of human surveillance systems, especially passive, are inadequate because the results are usually belated in order to introduce specific prophylactic measures.

The passive surveillance of the clinical cases in animal populations gives important veterinary medicine and public health data. During the first three seasons of WNV outbreaks in North America avian mortality proved to be extensive. Natural fatal infections have been confirmed based on the positive laboratory tests on over 28,000 carcasses of 198 bird species [17]. The surveillance system based on the monitoring of bird mortality has been proven as a valuable tool for assessing the risk of West Nile virus infection in humans and even in equines in North America [18]. On the contrary, during many WNV outbreaks in equine and human populations in Europe, an increased mortality in birds was not reported, so the passive surveillance in birds could only be a part of a part of more complex WNV surveillance system in Europe. Another important limiting factor of dead bird surveillance are the results that are clearly depending on carcasses submission by citizens and the public awareness of WNV importance.

The passive surveillance of horses appeared to be the most cost-effective system in the current European context [19], but it depends on the number and the presence of specialized veterinary clinics. That is the reason that in some countries and regions equine WNV clinical cases can go unrecognized. For these countries, like Croatia is, with very few specialized equine clinics, the passive surveillance of horses is inappropriate and the active surveillance apparently represents the best WNV surveillance system.

Due to mostly unspecific clinical signs even the neurological form of WNV infection in horses, could be misdiagnosed as some other disease without laboratory confirmation. There is even some possibility that Mraclin Disease described in Croatia in 1938, according to clinical signs was unrecognised outbreak of WNV infection in horses [20].

In Croatia, during the years 2010 and 2011 [21], the advantage of the active horse surveillance over the passive one was confirmed when WNV activity was observed in Eastern Croatia without any reported horse clinical case. Even more, in 2012 the first human WNV clinical cases occurred in the region where the highest seroprevalence in horses had been recorded in the previous two years, highlighting the importance of the active horse surveillance for public health data.

On the basis of the 2010 and 2011 surveillance results, in 2012 active WNV surveillance in the sentinel horses in Croatia was conducted in six counties with the highest WNV seroprevalence in horses confirmed in the previous two years.

MATERIALS AND METHODS

Between June and October 2012, 1804 horse sera were sampled for IgM WNV antibodies testing as the confirmation of an acute infection. Additionally, 1472 sera samples were tested for the presence of IgG WNV antibodies in the seroprevalence study. The animals, which were randomly selected, originated from six Croatian counties, namely: County of Brod-Posavina, County of Istra, County of Osijek-Baranja, County of Požega-Slavonia, County of Virovitica-Podravina and County of Vukovar-Srijem with the confirmed high WNV seroprevalence in horses in the previous two years.

The criterion for the selection of animals was that they were not moved in the international transport nor moved between counties. The age of animals was from six months to more than 25 years. The horses included in this study had no WNV vaccination history and at the time of sampling all animals were asymptomatic.

The horse serum samples were tested for WNV IgM antibodies using a commercial enzyme-linked immunosorbent assay (ID Screen West Nile IgM Capture, ID.VET, Montpellier, France) and for WNV IgG antibodies using a commercial competitive enzyme-linked immunosorbent assay c-ELISA (ID screen West Nile competition ELISA kit, ID.VET, Montpellier, France) in accordance with the manufacturer's instructions.

RESULTS

Study of acute WNV infection in horses

Out of 1084 horse sera samples tested for IgM WNV antibodies, 12 were positive (0,7%). Positive sera samples originated from the three counties. Six IgM positive samples were taken from horses on five different locations of County of Vukovar-Srijem from July 20th till August 1st. The seroprevalence of acute WNV infection in this county was 1,3%. The same seroprevalence of acutely infected horses was found in Virovitica-Podravina County with the confirmation of four acutely infected horses on three different locations. The horse sera were sampled during September 2012. Two more acute infections were confirmed in Brod-Posavina County, on different locations, with overall seroprevalence on county level of 0.9%. The sera were sampled during September 2012. In other three counties, Osijek-Baranja, Istra, Požega-Slavonia, acutely infected horses were not found. Horse sera from these counties were sampled from 15th June to 15th July in Osijek-Baranja County and during September 2012 in Istra and Požega-Slavonia counties (*Table 1, Figure 1*).

Table 1. The seroprevalence of IgG and IgM WNV antibodies in six Croatian counties included in WNV active horse serosurveillance during 2012

COUNTY	IgG			IgM		
	Number of samples	Number of positive samples	Seroprevalence (%)	Number of samples	Number of positive samples	Seroprevalence (%)
Brod-Posavina	184	32	**17,4**	216	2	**0,9**
Istra	276	5	**1,8**	177	0	**0,0**
Osijek-Baranja	257	26	**10,1**	360	0	**0,0**
Požega-Slavonia	276	6	**2,2**	283	0	**0,0**
Virovitica-Podravina	276	36	**13,0**	313	4	**1,3**
Vukovar-Srijem	203	23	**11,3**	455	6	**1,3**
Total	**1472**	**128**	**8,7**	**1804**	**12**	**0,7**

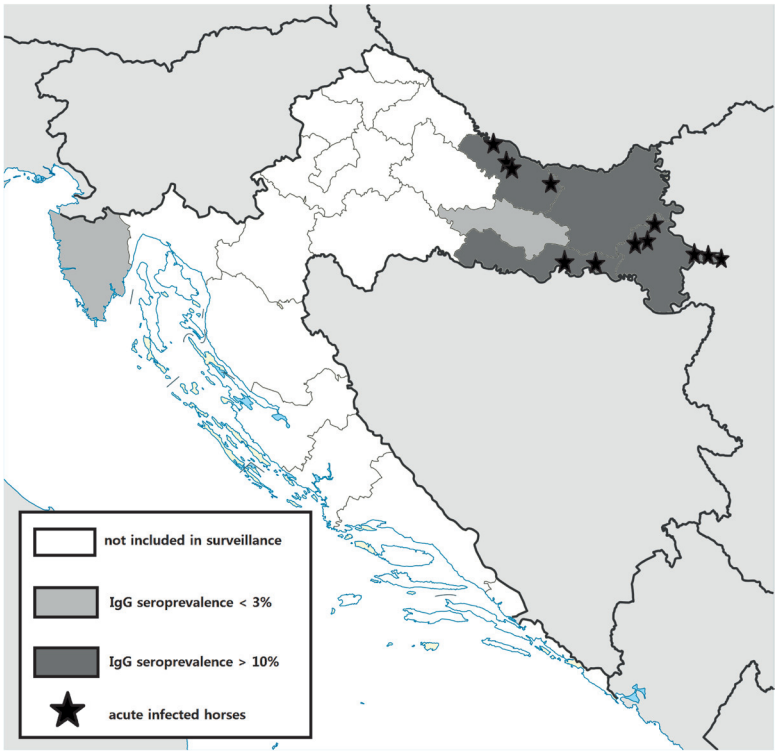

Figure 1. The seroprevalence of IgG WNV antibodies on county level and acutely infected horses during the transmission season 2012

WNV seroprevalence study in horses

In total, 1472 horse sera samples were tested for IgG WNV antibodies in the seroprevalence study for the transmission season 2012. The seroprevalences varied considerably between counties and the average seroprevalence was 8,7 %. The highest seroprevalence was found in County of Brod-Posavina with 32 out of 184 IgG positive samples (17,4%). Seroprevalence over 10% was found in three more counties. In County of Virovitica-Podravina 36 out of 276 horse sera samples were positive (13,0%), in County of Vukovar-Srijem 23 out of 203 tested samples (11,3%) were positive and finally, out of 257 horse sera samples 26 were positive in County of Osijek-Baranja (10,1%). Much lower seroprevalences were recorded in other two counties. County of Požega-Slavonia had only six out of 276 samples positive (2,2%) and Istra County five out of 276 (1,8%) (*Table 1, Figure 1*).

DISCUSSION

In Croatia, WNV serologically positive horses were detected more than 10 years ago [22] and active surveillance in horses was established in 2010. During the 2010 and 2011 the highest seroprevalence was observed in the same three counties in Eastern Croatia (County of Požega-Slavonia, County of Osijek-Baranja and County of Vukovar-Srijem) without significant differences in the seroprevalences on county level in these two consecutive seasons [21]. In this study we present high seroprevalences in sentinel horses, of more than 10%, in four out of six counties included in WNV surveillance during 2012. Like in the previous two years, the high seroprevalences were recorded in County of Osijek-Baranja, and in County of Vukovar-Srijem, but with an increase in 2012. Furthermore, high seroprevalence levels in the year 2012 were found in County of Brod-Posavina and County of Virovitica-Podravina with a drastic increase comparing to 2010 and 2011. On the contrary, the seroprevalence in Požega-Slavonia County was decreased in comparison with previous seasons, as well as the seroprevalence in County of Istra.. During 2012, the first evidence of WNV neuroinvasive clinical cases in humans were reported in Osijek-Baranja, Vukovar-Srijem and Brod-Posavina counties [23]. Many authors' studies noticed a correlation between WNV activity in animal population and the prediction of WNV human clinical cases occurrence [24,25,26]. The results of our study confirmed this correlation with the evidence of human neuroinvasive clinical cases in counties with high WNV seroprevalence in horses that is increasing.

Acute infections in horses were confirmed, for the first time in Croatia, during the surveillance in the transmission season 2012. Acute infection took places in the three counties with the increase of WNV seroprevalence. As referred in the outbre-

aks in other countries, acute infections in equids usually occurred for some time before the detection of WNV cases in humans [27,28]. The confirmation of acutely infected horses in County of Vukovar-Srijem, more than 2 weeks before the first human clinical cases occurred, highlights the importance of active horse surveillance as a predictive tool in public health in Croatia.

Acutely infected horses were also found in County of Brod-Posavina during the testing of horse sera sampled after the reporting of first human clinical cases. These results confirmed a high viral activity in this region in the transmission season 2012. In County of Virovitica-Podravina acutely infected horses were also confirmed on three distinct locations but with no evidence of human clinical cases. Lack of human cases could easily be the result of not reporting and of insufficient awareness of physicians because this county was not considered as the region of high risk. On the contrary, County of Osijek-Baranja had four human clinical cases with no confirmed acute infections in horses. It should be kept in mind that the time of sera sampling in this county had been before the peak season of vector activity so the the possibility of acute infections in horses had been very low.

The absence of reported equine clinical cases during the transmission season 2012, despite high seroprevalence, confirmed acute infection in horses and human clinical cases could be the result of the different virulence of Croatian WNV field strains for horses and humans. It is more likely that the absence of reported equine clinical cases is the result of not reporting or not recognizing WNV infection related symptoms in horses. Anyway, the absence of reported clinical cases emphasises the deficiencies of WNV passive surveillance in horses in Croatia.

In conclusion, the results of the active serosurveillance in the horse population in Croatia during the year 2012 showed the increase of WNV activity in the eastern part of Croatia, the same area where the viral activity had been detected in the previous two years. The increase in seroprevalence and, for the first time, the evidence of acute infections in horses was observed in the same region where human WNV clinical cases were reported afterwards. The results confirmed the active serosurveillance system in horses as the most suitable way of WNV surveillance in Croatia. Collecting data through the active serosurveillance in horses represents an excellent WNV early warning system and could be used as a guideline for the establishment of adequate control measures in public health.

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Sažetak

Serološko istraživanje infekcije virusom Zapadnog Nila u konja u Hrvatskoj 2012. godine

Tijekom 2012. godine praćenje proširenosti infekcije virusom Zapadnog Nila u Republici Hrvatskoj provođeno je kontrolom proširenosti infekcije u asimptomatskih konja. Tijekom sezone prijenosa, od lipnja do listopada 2012. godine, pretražena su 1.804 uzorka seruma konja na prisutnost IgM specifičnih protutijela u svrhu dokazivanja akutnih infekcija virusom Zapadnog Nila. Povrh toga, radi određivanja seroprevalencije, pretražena su 1.472 uzorka seruma konja na prisutnost IgG protutijela. Programom je bilo obuhvaćeno šest županija Republike Hrvatske. Rezultati istraživanja pokazali su porast seroprevalencije u četiri županije istočne Hrvatske, dok su akutno zaražene životinje dokazane u tri županije istog područja. Tijekom istog razdoblja zabilježeni su prvi klinički slučajevi bolesti Zapadnog Nila u ljudi na području županija s dokazanim porastom seroprevalencije u konja. Akutne infekcije konja dokazane su dva tjedna prije prvih kliničkih slučajeva u ljudi. Prikazani rezultati potvrđuju da je provođenje aktivne kontrole konja na prisutnost infekcije virusom Zapadnog Nila iznimno važno za kontrolu te bolesti u Republici Hrvatskoj, kako za veterinarsku medicinu, tako i za javno zdravstvo.

Ključne riječi: virus Zapadnog Nila; seroprevalencija; konji; javno zdravstvo.

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