

## WEST NILE VIRUS MONITORING IN WILD BIRDS IN SLOVENIA

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### Summary

Wild bird carcasses were collected through passive monitoring of wild bird mortality. In necropsy, brain samples were taken from dead wild birds in 2010, 2011 and 2012. Samples were used for detection of West Nile virus by reverse transcriptase-polymerase chain reaction (RT-PCR). No WNV nucleic acid was detected by RT-PCR. In the light of the data presented there is no evidence of wild bird mortality in Slovenia due to WNV activity.

**Keywords:** West Nile virus; wild birds; Slovenia.

### INTRODUCTION

The presence of West Nile virus (WNV) in Europe has been known for decades. Virus transmission is related to the ornithophilic mosquitos. Wild birds are an important natural amplifying host for the virus. Humans, horses, and some other mammals are considered dead-end hosts [1]. Cases of the West Nile disease (WND) in humans and horses were reported in Europe and from Mediterranean basin [1,2,3]. In Europe, wild bird mortality related to WNV infection was only seen very seldom [1,3]. Recently, WNV was detected in dead wild birds in Hungary in 2004 [4]. Since then the spread of the virus in wild birds was observed in Hungary, Austria [5], Italy [6] and Greece [7]. In Slovenia, the presence of WNV was reported on the basis of serological studies on forest workers [8], horses [9] and wild birds [10]. Birds testing positive were found among passerines and free-living domestic pigeons [10,11,12].

The main goal of our study was to obtain further information about the role of wild birds in the ecology of WNV in Slovenia.

## MATERIALS AND METHODS

### Samples

From 2010 to the end of 2012, wild birds of various orders were collected as part of the passive surveillance program for detection of avian influenza viruses. Birds were dissected, and brain specimens stored at -70 C° until nucleic acid extraction was performed.

### Molecular analysis

Viral RNA was extracted from the brain homogenates by using QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany) with an input volume of 140 µl and elution volume of 60 µl, in accordance with the manufacturer's instructions.

For detection of the viral RNA, reverse transcriptase-polymerase chain reaction (RT-PCR), based in the conserved non-structural protein 5 (NS5) was used for its ability to detect all members of the Japanese encephalitis virus antigenic group of flaviviruses [13]. RT-PCR was performed with Qiagen One-Step RT-PCR kit by using 2 µl RNA and 16pmol of forward primer: 5'-GARTGGATGACVACRGAAGACATGCT-3' and reverse primer: 5'-GGGGTCTCCTCTAACCTCTAGTCCTT-3' in a 20 µl total reaction volume.

The RT-PCR products were analysed by electrophoresis in a 1.8% agarose gel stained with ethidium bromide.

## RESULTS

In behalf of passive monitoring of wild bird mortality in 2010, 2011 and 2012 a total of 58 brain tissue samples were taken during a routine necropsy from 15 different species of wild birds (*Table 1*). All samples were screened for the WNV nucleic acid using RT-PCR. All 58 samples tested for the presence of the West Nile viral nucleic acid by RT-PCR were negative.

**Table 1.** The number of wild birds sampled and tested

Species of wild birds		Number of wild birds sampled/ tested		
		2011	2012	2013
Common Buzzard	<i>Buteo buteo</i>	6	3	1
Mallard	<i>Anas platyrhynchos</i>	1	3	2
Mute Swan	<i>Cygnos olor</i>	7	1	10
White Stork	<i>Ciconia ciconia</i>	5	1	-
European Sparrowhawk	<i>Accipiter nisus</i>	1	-	-
Common Pigeon	<i>Columba livia</i>	1	2	-
Common Kestrel	<i>Falco tinnunculus</i>	2	-	-
Barn Swallow	<i>Hirundo rustica</i>	1	-	-
Blackbird	<i>Turdus merula</i>	-	1	3
Gull	<i>Larus spp.</i>	-	-	1
Grey Heron	<i>Ardea cinerea</i>	-	-	1
Hooded Crow	<i>Corvus corone cornix</i>	-	-	2
Hawfinch	<i>Coccothraustes coccothraustes</i>	-	-	1
Greenfinch	<i>Carduelis chloris</i>	-	1	-
Common Starling	<i>Sturnus vulgaris</i>	-	-	1
<b>Total</b>	<b>15 species</b>	<b>24</b>	<b>12</b>	<b>22</b>

## DISCUSSION

In our study, all 58 wild birds tested negative by RT-PCR for the presence of the WNV nucleic acid. There was no evidence of wild bird mortality due to WNV activity in Slovenia. To our knowledge, WNV infections in free-living birds in Slovenia have never been associated with clinical symptoms or mortality (personal communication), although outbreaks and sporadic cases of the West Nile disease in wild birds were reported from the neighbouring countries [4,5,14]. In Europe, a fatal WNV infection in wild birds was first observed in Hungary in 2004, in a Goshawk (*Accipiter gentilis*) fledgling [4]. The isolated virus belonged to the lineage 2 WNV and was seen for the first time in Europe. Since then, passive monitoring of wild bird mortality in Hungary and Austria in 2008 and 2009 revealed that Goshawks, Sparrow hawks (*Accipiter nisus*), and other birds of prey were most commonly affected [5]. In Italy, a WNV strain belonging to lineage 2 was for the first time detected in the tissues of a Wild Collared Dove (*Streptopelia decaocto*) found dead in 2012 [6]. Moreover, WNV lineage 1 tested positive in European Magpie (*Pica pica*) and Eurasian Jay (*Garrulus glandarius*) during active surveillance in the same country in 2010

[14]. The reason of the explosive spread of the lineage 2 virus in Central Europe remains yet unclear [5]. It is possible that the virus was introduced to Hungary by the migratory birds [1,4,5]. The vertebrate reservoir host (e.g. free-living pigeons) is hypothesized for being responsible for the dissemination of the virus. In Hungary, specific antibodies were found in 70 % of pigeons [5]. In Italy, a WNV lineage 1 was isolated from pools of brain, kidneys, heart and spleen of one pigeon [15]. Among the resident species Magpies, Carrion Crows and pigeons are the most probable species involved in the endemic cycle of WNV [3]. In our country, the prevalence of antibodies against WNV in free-living Domestic Pigeons (*Columba livia*) was 12.4 % (23/186). Pigeons were caught and sampled at different locations in the city of Ljubljana, the capital of Slovenia [11]. Pigeons seem to be particularly suitable reservoirs and the infection of urban pigeons might increase the risk of human infections [5].

Considering reports of the increased WNV activity in some European countries further systematic research is needed to understand the epidemiology and the ecology of WNV in Slovenia.

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#### Sažetak

### **Praćenje virusa Zapadnog Nila u divljih ptica u Sloveniji**

Lešine divljih ptica sakupljene su zbog praćenja mortaliteta. Uzorci mozga uginulih ptica bili su uzimani pri razudbi tijekom 2010., 2011. i 2012. godine te su bili pretraženi na virus Zapadnog Nila lančanom reakcijom polimerazom uz prethodnu reverznu transkripciju (RT-PCR). Nukleinska kiselina za virus Zapadnog Nila nije bila dokazana RT-PCR-om. Na osnovi tih rezultata može se ustvrditi da aktivnost virusa Zapadnog Nila nije dokazana u divljih ptica u Sloveniji.

**Ključne riječi:** virus Zapadnog Nila; divlje ptice; Slovenija.

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