



The Complete Sequence of a West Nile Virus Lineage 2 Strain Detected in a *Hyalomma marginatum marginatum* Tick Collected from a Song Thrush (*Turdus philomelos*) in Eastern Romania in 2013 Revealed Closest Genetic Relationship to Strain Volgograd 2007

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Abstract

In this study the first complete sequence of the West Nile virus (WNV) lineage 2 strain currently circulating in Romania was determined. The virus was detected in a *Hyalomma marginatum marginatum* tick collected from a juvenile song thrush (*Turdus philomelos*) in the Romanian Danube Delta close to the city of Tulcea, end of August 2013. Our finding emphasizes the role of ticks in introduction and maintenance of WNV infections. Sequence analyses revealed close genetic relationship of the Romanian WNV strain to strain Reb_Volgograd_07_H, which was isolated from human brain tissue during an outbreak of West Nile neuroinvasive disease (WNND) in Russia in 2007. In 2010 the Eastern European lineage 2 WNV caused an outbreak of human WNND in Romania. Partial sequences from subsequent years demonstrated that this WNV strain became endemic in Eastern Europe and has been causing outbreaks of varying sizes in southern Russia since 2007 and in Romania since 2010.

Citation: Kolodziejek J, Marinov M, Kiss BJ, Alexe V, Nowotny N (2014) The Complete Sequence of a West Nile Virus Lineage 2 Strain Detected in a *Hyalomma marginatum marginatum* Tick Collected from a Song Thrush (*Turdus philomelos*) in Eastern Romania in 2013 Revealed Closest Genetic Relationship to Strain Volgograd 2007. PLoS ONE 9(10): e109905. doi:10.1371/journal.pone.0109905

Editor: Lark L. Coffey, University of California Davis, United States of America

Received: July 15, 2014; **Accepted:** August 29, 2014; **Published:** October 3, 2014

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

Funding: This study was partially supported by the European Union grants FP7-261504 EDENext (www.edenext.eu) to JK, MM, BK, VA, and NN as well as FP7-261391 EuroWestNile (www.eurowestnile.org) to JK and NN. Funder of both grants: European Commission. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Mosquitoes, primarily of the genus *Culex*, are considered the main vectors of West Nile virus (WNV), a zoonotic member of the genus *Flavivirus*. Wild birds constitute the principal hosts of the virus amplifying it in a bird-mosquito cycle. Certain 'bridge' mosquito species have been determined to transmit the virus to humans and other mammals, which are regarded dead-end hosts [1], [2]. The role of ticks as WNV vectors had been poorly investigated to date [3].

Romania has a long-standing history of WNV infections, including severe outbreaks of human West Nile neuroinvasive disease (WNND) in 1996 with 393 confirmed cases [4], and in 2010 with 57 cases. Affected patients were distributed among 19 districts in the southern, western, central and eastern parts of the country [5]. The '2010' WNV strain became endemic and has been the cause of outbreaks of varying sizes each following year (http://www.ecdc.europa.eu/en/healthtopics/west_nile_fever/Pages/epidemiological_updates.aspx; http://www.ecdc.europa.eu/en/healthtopics/west_nile_fever/West-Nile-fever-maps/Pages/historical-data.aspx). Nevertheless, the complete sequence

of the main WNV strain circulating in Romania since 2010 has not been determined as yet. Therefore goals of this study were to determine the complete genomic sequence of the WNV strain currently circulating in Romania, assess its pathogenicity and neuroinvasive markers, investigate its phylogenetic relatedness to other WNV strains, and discuss the role of ticks in WNV introduction and maintenance.

Materials and Methods

A total of 32 ticks were found randomly on a total of 23 birds, which had been captured using mist nets [6] in the Danube Delta Biosphere Reserve, Romania. They were investigated for the presence of WNV within the framework of the European Union FP7 project EDENext. Specifically, the birds were captured at the following locations: Enisala (44°53'28.28"N/28°49'50.97"E), Gr. Lupilor (44°41'46.82"N/28°56'15.70"E), Salcioara (44°47'55.30"N/28°53'57.57"E), Maliuc Mila (45°10'34.61"N/29°3'53.91"E), Rachitarie (45°11'34.74"N/29°5'8.16"E), and Maliuc (45°10'35.96"N/29°6'29.51"E). All ethical and research certifications, approvals and permits were issued by the responsible