

Brief Summary of Ph.D. Thesis

West Nile Virus in horses in Hungary: epidemiology, diagnostic and prevention technics

Dr. Orsolya Eszter Fehér

Supervisor: Dr. Orsolya Korbacska-Kutasi



UNIVERSITY OF VETERINARY MEDICINE

Doctoral School of Veterinary Science

Budapest

2022

Supervisor:

Dr. Orsolya Korbacska-Kutasi

Associate Professor

University of Veterinary Medicine Budapest

Supervisor

This brief summary has been written in 8 copies.

This is the copy.

Dr. Fehér Orsolya Eszter

Introduction

West Nile virus (WNV) is a globally emerging pathogen belonging to the genus *Flavivirus* in the family *Flaviviridae*. The arthropod-borne single stranded RNA virus is a member of the Japan encephalitis virus complex (JEV). WNV was first detected in the West Nile province of Uganda.

According to phylogenetic analysis, eight divergent WNV lineages have been described. WNV lineages 1 and 2 are the most widespread and have caused most of the major epidemics encountered so far. Mosquitoes transmit this zoonotic arbovirus between wild birds (natural hosts) and other vertebrates. Horses and humans are incidental, dead-end hosts but can develop severe neurological disorders.

In Hungary, WNV caused the first outbreak in 2003 in a flock of geese and in 2004 in

goshawks. WNV is an emerging pathogen in Hungary, causing severe outbreaks in equines and humans since 2007. Among the many flaviviruses, which can cause disease in mammals, the WNV has the most impact on equid health. In the last decade in Hungary and the neighboring countries, West Nile neuroinvasive Disease (WNND) has been caused in dramatically increasing numbers by lineage 2 WNV strains both in horses and in humans.

The examination of WNV infections in horses has a special role because the susceptibility of the species exceeds human sensitivity, so equids play an important role in monitoring the pathogen as an indicator species.

Objectives

1. Our first study aimed to provide a comprehensive report on the clinical signs of West Nile Neuroinvasive Disease (WNND) in horses in Hungary.
2. Our objective in the second study was to compare the findings from the cerebrospinal fluid (CSF) samples of horses with WNND with those of healthy controls.
3. In the third study, we summarize the clinical diagnostic and treatment features of WNND specific to Hungary and describe two cases of WNND with a particular focus on how recumbent neurological cases could be managed in stable conditions.

4. The aim of our fourth study was to characterize the serum-neutralizing (SN) antibody titers against a lineage 2 WNV strain in response to vaccination with an inactivated lineage 1 vaccine (Equip® WNV).

Materials and Methods

In the first study, we examined the details of WNV infections officially diagnosed by the National Food Chain Safety Office (NÉBIH) between 2007 and 2020. Temporal and spatial data were analyzed for all horses that tested positive for IgM ELISA. The clinical details of acute neuroinvasive diseases (WNND) were summarized according to a uniform study protocol. We subjected the data to descriptive statistical tests and looked for correlations between certain symptoms, treatment methods and the outcome of the disease.

In the second study, cerebrospinal fluid samples were collected from 13 horses with WNND during atlanto-occipital and/or lumbosacral puncture. The liquor samples were subjected to cytological, biochemical and serological tests. We compared our results with

the parameters of samples from 20 healthy control horses.

In the third study, we present the disease treatment options through a case study of two horses suffering from neuroinvasive disease caused by WNV.

Within the framework of the fourth study, we performed a serological survey using an IgG ELISA test involving 82 horses in a stud in Hungary, and then selected 32 of the seronegative horses for the research. We included 22 horses in the vaccinated group and 10 horses in the control group. Using a virus neutralization method, we examined the immune response induced by the vaccine based on lineage 1 on days 49, 211, 393, 408, 576, 667 and 758 of the research.

Results

Collecting the details of a total of 124 clinical cases, and starting from the initial sporadic diseases, the entire country became endemic by the end of the research period. There was no considerable relationship between the occurrence of WNND and age, breed, or gender. Ataxia was the most common neurological sign related to the disease, but weakness, behavioral changes, and muscle fasciculation appeared frequently. Apart from recumbency combined with inappetence, no other clinical sign or treatment regime correlated with survival.

Protein, particular enzymes, ions, glucose, and lactate showed abnormal levels in a significant number of WNV cases. None of the six horses with elevated blood glucose concentrations survived. There was more neutrophilic than

mononuclear pleocytosis identified with WNV infection. The CSF findings of WNV encephalomyelitis patients are non-specific and variable but facilitate the differential diagnosis.

Mean antibody titers against lineage 1 WNV were significantly higher in the vaccinated group compared to the control group at all-time points after the primary vaccination dose. Serum neutralizing (SN) antibody titers appeared significantly higher against lineage 1 than lineage 2 at all-time points. Similarly, mean antibody titers against lineage 2 WNV were significantly higher in the vaccinated group compared to the control group at all time points. According to the results, vaccination with an inactivated lineage 1 vaccine induces the production of antibodies against both WNV lineages 1 and 2 strains up to 2 years after booster vaccination.

Conclusions

West Nile virus has been an endemic pathogen in Hungary in the last 14 years, causing severe outbreaks among the equine and human population all over the country. According to the higher susceptibility, the examination of equines may play an important role in predicting outbreaks. An overall surveillance system in Hungary that includes both disease and PCR screening results would provide important information for both veterinary and human medicine. WNV infection is recognized on time, and a well-organized supported therapy is an essential factor of the successful outcome of the disease. During the vaccine tests, we prepared a suitable vaccination recommendation especially to lineage 2 endemic areas. Future studies may use equine cases as an indicator of WNV-intensive

transmission activity and epidemiological and entomological studies to further understand the risk factors of WNV epidemic transmission. The collective results of this research can give a comprehensive overview of the Hungarian equine WNND cases and serve as the base point for collaborative inter-discipline research and programs on West Nile virus. Our work also reflects the urgent need for a national, organized surveillance system related to West Nile virus in the One Health approach.

New scientific results

1. Our study is the first comprehensive and descriptive review containing a high number of horses infected by lineage 2 West Nile virus resulting in West Nile neuroinvasive Disease.
2. We describe the temporal and spatial spread dynamics of the West Nile virus lineage 2 in Hungary covering a 14 years of data collection period.
3. Our study is the first one to describe in detail the characteristics of the cerebrospinal fluid of horses with West Nile neuroinvasive Disease caused by a lineage 2 strain.
4. These studies describe and evaluate treatment protocols and clinical signs in connection with their prognostic values.

5. Evaluation of the most common inactivated Lineage 1 vaccine in Europe from the point of view of virus neutralizing titers and, based on these results, determination of an applicable vaccination plan in the geographical region characteristic of Central Europe.

Publications based on the results of the PhD dissertation

a) Publications published in foreign scientific journal with an impact factor

Fehér, O., Bakonyi, T., Barna, M., Nagy, A., Takács, M., Szenci, O., Joó, K., Sárdi, S., & Korbacska-Kutasi, O. (2020). **Serum neutralizing antibody titers against a lineage 2 neuroinvasive West Nile Virus strain in response to vaccination with an inactivated lineage 1 vaccine in a European endemic area.** *Veterinary Immunology and Immunopathology*,227,110087.

<https://doi.org/10.1016/j.vetimm.2020.110087>

Fehér, O. E., Fehérvári, P., Tolnai, C. H., Forgách, P., Malik, P., Jerzsele, Á., Wagenhoffer, Z., Szenci, O., Korbacska-Kutasi, O. (2022). **Epidemiology and Clinical Manifestation of West Nile Virus Infections of Equines in Hungary, 2007–2020.** *Viruses*,14(11),2551.

<https://doi.org/10.3390/v14112551>

Kutasi, O., **Fehér, O.**, Sárdi, S., Balogh, N., Nagy, A., Moravszki, L., Bódai, E., & Szenci, O. (2020). **Characterisation of the cerebrospinal fluid of horses with West Nile virus neuroinvasive disease.** *Acta Veterinaria Hungarica*, 68(2), 177–185.,

<https://doi.org/10.1556/004.2020.00022>

Zana, B., Erdélyi, K., Nagy, A., Mezei, E., Nagy, O., Takács, M., Bakonyi, T., Forgách, P., Korbacska-Kutasi, O., **Fehér, O.**, Malik, P., Ursu, K., Kertész, P., Kepner, A., Martina, M., Süli, T., Lanszki, Z., Tóth, G. E., Kuczmog, A., Kemenesi, G. (2020). **Multi-Approach Investigation Regarding the West Nile Virus Situation in Hungary, 2018.** *Viruses*,12(1),123.

<https://doi.org/10.3390/v12010123>

Joó, K., Bakonyi, T., Szenci, O., Sárdi, S., Ferenczi, E., Barna, M., Malik, P., Hubalek, Z., **Fehér, O.**, & Kutasi, O. (2017). **Comparison of assays for the detection of West Nile virus antibodies in equine serum after natural infection or vaccination.** *Veterinary Immunology and Immunopathology*, 183,1–6.

<https://doi.org/10.1016/j.vetimm.2016.10.015>

b) Publications published in Hungarian scientific journal with an impact factor

Fehér, O., Szoboszlai, H., Korbacska-Kutasi, O.: **Treatment of West Nile Virus caused encephalomyelitis in horses at stable conditions: Case study**, *Hungarian Veterinary Journal*, 2019. (141.), 4. sz., 195-206. p.

b) Conference oral publications

Dr. Fehér Orsolya Eszter, Dr. Malik Péter, Dr. Szögyényi Zsuzsanna, Dr. Halas Máté, Dr. Bakonyi Tamás, Dr. Joó Kinga, Prof. Dr. Szenci Ottó, Dr. Korbacska-Kutasi Orsolya: A Nyugat-nílusi vírus magyarországi előfordulásával kapcsolatos tapasztalatok lovakban, XXVI. Lógyógyászati Kongresszus, Mátraháza, 2018.

Dr. Fehér Orsolya Eszter, Dr. Malik Péter, Dr. Szögyényi Zsuzsanna, Dr. Halas Máté, Dr. Bakonyi Tamás, Dr. Joó Kinga, Prof. Dr. Szenci Ottó, Dr. Korbacska-Kutasi Orsolya: A Nyugat-nílusi vírus magyarországi előfordulásával kapcsolatos tapasztalatok lovakban, MAOK Hajdú-Bihar megyei Éves Továbbképzése, Hajdúszoboszló, 2018.

Dr. Fehér Orsolya: A Nyugat-nílusi vírus magyarországi előfordulásával kapcsolatos tapasztalatok lovakban, MTA ÁLLATORVOS-TUDOMÁNYI BIZOTTSÁGA ÁTE ÁLLATORVOSTUDOMÁNYI DOKTORI ISKOLA AKADÉMIAI BESZÁMOLÓK (2019).

Fehér Orsolya Eszter, Forgách Petra, Marosi András, Malik Péter, Nagy Anna, Takács Mária, Korbacska-Kutasi Orsolya: Nyugat-nílusi vírus és más flavivírusok okozta fertőzések szerológiai vizsgálata, XXVII. Lógyógyászati Kongresszus, Balatonkenese, 2019.

Fehér Orsolya Eszter, Forgách Petra, Marosi András, Malik Péter, Nagy Anna, Takács Mária, Korbacska-Kutasi Orsolya: A Nyugat-nílusi vírus és más flavivírusok aktivitása Magyarországon, MTA ÁLLATORVOS-TUDOMÁNYI BIZOTTSÁGA ÁTE ÁLLATORVOSTUDOMÁNYI DOKTORI ISKOLA AKADÉMIAI BESZÁMOLÓK (2020).

Fehér Orsolya Eszter, Piller Pálma, Forgách Petra,
Marosi András, Korbacska-Kutasi Orsolya: A
Nyugat-nílusi vírus okozta megbetegedések során
kialakuló immunválasz vizsgálata lovakban, MTA
ÁLLATORVOS-TUDOMÁNYI BIZOTTSÁGA ÁTE
ÁLLATORVOSTUDOMÁNYI DOKTORI ISKOLA
AKADÉMIAI BESZÁMOLÓK (2021).

Acknowledgements

First, I am thankful to my supervisor, friend, and mentor, Dr. Orsolya Korbacska-Kutasi. Her support and encouragement helped me along the gradual and doctoral studies, and I am grateful to her for providing me with the West Nile virus topic.

I warmly thank all the people working at the Veterinary University Budapest, Institute for Animal Breeding, Nutrition, and Laboratory Animal Science, and at the Virology Department.

I am deeply grateful to the colleagues of the Department of Virology at the National Public Health Center. I could always ask my questions and received endless support from the whole team during the laboratory work.

I want to thank all my co-authors for the publishing and cooperation opportunities. I

would like to thank all contributed field veterinarians and horse owners for providing me with information and samples for the research.

I owe my warmest gratitude to my family, and friends, who have never said no to any impossible situation, and their endless support during my Ph.D. work.