

## SEROPREVALENCE OF WEST NILE FEVER VIRUS IN HORSES IN THE BELGRADE EPIZOOTIOLOGICAL AREA

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### Abstract

West Nile fever is a vector borne viral disease that can affect humans, horses, birds and sometimes other species of animals. Every year West Nile fever is detected in the human population in Serbia. The disease often occurs in a subclinical form, but most clinically evident cases occur in horses. Therefore, horses are recommended as a sentinel species for monitoring the general incidence of West Nile fever in a specific territory. Our goal was to determine the prevalence of antibodies against West Nile fever virus in horses in the Belgrade epizootiological area. We examined serum samples from 77 horses to determine the seroprevalence of West Nile fever virus in horses throughout the city of Belgrade. Sera were tested by commercial ELISA tests for detection of specific IgG-class antibodies to West Nile fever virus and for the detection of specific IgM-class antibodies to confirm the presence of old and acute (recent) infections in horses. The results confirmed that West Nile fever virus is widespread, detected in 70.1% of the surveyed horse population in Belgrade, and we also detected 5.1% of acute cases had occurred due to horses being infected in 2019. The seroprevalence of West Nile virus in the horse population in the municipality of Belgrade is increasing.

**Key Words:** IgG antibodies, IgM antibodies, ELISA test, West Nile disease, vector borne disease, Belgrade

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## INTRODUCTION

West Nile fever is a vector-borne zoonosis of viral aetiology that can affect horses, birds and sometimes other species of animals besides humans (Stelle et al., 2000). Antibodies against West Nile fever virus have been detected sporadically in sera of other animal species such as dogs, cats, bats, squirrels, deer, sheep, alpacas, llamas, camels and more (Durand et al., 2012; OIE Ter. Manual, 2019). The first West Nile virus strain was isolated from a human patient in Uganda in 1937 (Bakonyi et al., 2006). The first reported outbreak of West Nile virus infection in humans in Serbia was detected in 2012 (Popović et al., 2013). No antivirals or other drugs are known to be effective in the prevention or treatment of West Nile virus infection (Campbell et al. 2002).

West Nile fever is now spreading by vectors very quickly and has become especially topical in our country and the region over the last decade. The virus is transmitted by hematophagous insects, most commonly mosquitoes of the genus *Culex* (Petrović et al., 2014). The virus circulates between birds and mosquitoes in which the virus can multiply, while infected horses and humans are a dead end (Zeller et al. 2004) without further transmission of virus. In support of this fact, Bunning examined mosquitoes that fed on the blood of viremic horses and found the virus was not transmitted to mosquitoes (Bunning et al., 2010). The disease is seasonal in nature, and in the northern hemisphere, West Nile fever occurs in the summer months from May or June to October, when the vectors are also abundant (Napp et al., 2018). Horses can be considered a sentinel species for the spread of West Nile fever (Petrovic et al. 2014; Beck et al., 2017).

The neuropathological pattern caused by West Nile fever in horses is usually mild to moderate, nonsuppurative polioencephalomyelitis (Snook et al., 2001). Viraemia in horses has a short duration and low intensity (Beck et al., 2017), so virus isolation by laboratory diagnostic methods for detection of virus or genome can sometimes be uncertain. Among all recommended serological assays for West Nile fever, ELISA tests for detection of IgG- or IgM-class antibodies are considered to be the most reliable methods. Detection of West Nile virus-specific IgG-class antibodies is evidence of old previous infection. The presence of specific IgM-class antibodies is evidence of an acute phase of the disease or recent infection. Although IgM-class antibodies in some percentage of people can be maintained in their circulatory system for more than a year, no such case has been detected in horses so far. In equids, West Nile virus-specific IgM antibodies are secreted as early as 8 days post infection (dpi) and can be detected up to  $70\pm 90$  dpi (Beck et al. 2017). Around 10% of infected horses and 1% of infected people manifest clinical symptoms of the disease (Castillo Olivares et al. 2011; Petrovic et al. 2015). Most infected individuals recover from West Nile fever without any symptoms in the subclinical form of the disease. However, the mortality rate is more than 50% for clinically ill horses (Petrović et al. 2015).

The aim of this study was to determine the presence of antibodies against West Nile virus in horses in the Belgrade epizootiological area, where the disease sporadically occurs every year in the horse and human population.

## **MATERIALS AND METHODS**

We sampled and tested the sera from 77 horses to detect specific IgG- and IgM-class antibodies against West Nile fever virus. Because West Nile fever has a seasonal character, horses' blood was sampled during the summer months to identify the number of recently infected individuals. The horses tested were of different ages. Nineteen horses originated from two large farms, while the other 58 horses were owned by individual horse owners who owned up to 2 horses.

Specific IgG-class antibodies were detected by a commercial ELISA assay for IgG class antibodies, Ingezim West Nile compact (Ingenasa, Spain) and the test was performed according to the manufacturer's instructions. This kit is based on a blocking immunoassay with plates coated with inactivated viral antigen. If the sample contains specific antibodies against West Nile virus, they will bind to the antigen absorbed on the plate. Next, specific monoclonal antibody conjugated with peroxidase was added. If the serum contains specific antibodies, they will not permit the labelled monoclonal antibody to bind to the antigen. After washing the plate to eliminate all non-fixed material, the presence or absence of labelled monoclonal antibody was detected by adding a substrate (TBM) and developing a colorimetric reaction. Specific IgM-class antibodies were detected by a commercial ELISA assay, Ingezim West Nile IgM (Ingenasa, Spain). This kit is based on a capture enzymatic immunoassay. A monoclonal antibody specific for equine IgM is fixed on a solid polystyrene microplate, with each serum sample placed into two wells. If the serum contains IgM antibodies, they will be captured by the monoclonal antibody absorbed on the plate. After washing, viral antigen was added to one well and control negative antigen (CNA) to the second well. If the serum contains specific IgM for West Nile virus, they will be captured. After washing, peroxidase conjugated monoclonal antibody specific for West Nile virus E protein was added. After final washing and addition of the substrate, a colorimetric reaction was developed. The OD obtained with negative antigen was subtracted from the OD obtained with positive antigen in order to interpret the results.

## **RESULTS AND DISCUSSION**

Out of 77 horse sera examined by the ELISA test, 54 sera (70.1%) were positive, while 23 sera (29.9%) were negative for the presence of specific IgG-class antibodies against West Nile virus (Table 1). From 77 horse sera examined by the ELISA test for the presence of West Nile fever specific IgM-class antibodies, 4 sera (5.1%) were IgM positive, while 73 horse sera (94.9%) were negative.

**Table 1.** West Nile virus antibodies, IgG- and IgM-class, detected by ELISA in sera from horses in the Belgrade area

Number of sera	IgG positive	IgG negative	% IgG positive	% IgG negative
77	54	23	70.1	31.1
Number of sera	IgM positive	IgM negative	% IgM positive	% IgM negative
77	4	73	5.1	94.9

Specific IgM-class antibodies in horses can be detected up to  $70\pm 90$  dpi and there are no exceptions, as is the case in humans, where more than 12% of the human population carry specific IgM-class antibody for more than a year (Papa et al., 2015). Therefore, the presence of West Nile virus-specific IgM-class antibodies in horse serum is always considered as confirmation of a recent or acute infection, with disease onset that occurred in the last season when serum was sampled. (Beck et al., 2017; Danis et al., 2011).

It should be kept in mind that West Nile fever occurs in clinical form in about 8 to 10% of infected horses and has a mortality rate of up to 50% (Angenvoort et al., 2013; Petrović et al., 2018). In other infected horses, the disease passes as a subclinical infection without symptoms. Considering the very short viraemia of low intensity in horses, a clear advantage in laboratory diagnostics is given to serological analyses. The IgM capture ELISA is useful for detecting equine antibodies resulting from recent natural exposure to West Nile virus, while IgG indirect and competitive ELISAs are suitable methods for surveillance to determine prevalence of infection (OIE Ter. Manual, 2019). Tests for detection of IgM-class specific antibodies for West Nile virus are highly specific, and false positive results caused by infection with other vector borne diseases, such as Usutu virus, do not occur (Back et al., 2017).

A few years ago, 30% of horses in Serbia had West Nile virus IgG-class antibodies, i.e., evidence of past infection, at least once in their lifetimes (Medić et al. 2014). Such a high percentage of West Nile virus-positive horses in the Belgrade area (more than 70%) determined in the current study is alarming for horse owners and for professionals dealing with West Nile fever in any aspect. Several types of West Nile fever vaccines for veterinary use in horses have been registered (Seino et al., 2007), and vaccination of horses in Serbia should be considered as a specific preventive measure in the future.

The first serological evidence of West Nile fever in horses in Serbia, including from the Belgrade region, was published 2011 (Lupulovic et al., 2011). Extensive monitoring of West Nile virus seroprevalence in horses was conducted in 2009, covering 12% of horses in the examined territory, and the seroprevalence in the Belgrade area was 35% (Petrović et al., 2015). There is an obvious trend of increasing seroprevalence, because our current results from 2019 showed a seroprevalence of 70.1% in horses in the Belgrade area. Previous seroprevalences for West Nile virus in horses in Serbia were 28.6% in 2011, 49.2% in 2012. and 46.9% in 2013 (Petrović et al., 2015). Obviously, the seroprevalence in the Belgrade area is much higher than the seroprevalence in

Serbia, which correlates with the results of the first extensive monitoring, when horses in the Belgrade region had a seroprevalence almost three times higher than the West Nile virus seroprevalence in horses in Serbia.

Our result of 5.1% seroprevalence for specific IgM-class antibodies in horses is much higher than in 2014, when the seroprevalence in the Belgrade area was 2.84%, while the seroprevalence in Serbia was 0.53% (Petrović et al. 2018). Compared with previous results, a trend of increasing seroprevalence for West Nile virus IgM in horses in the Belgrade area has been confirmed. This seroprevalence in horses living around the capital city has always been higher than the seroprevalence in Serbia.

## **CONCLUSION**

The seroprevalence for West Nile virus in the horse population in the Belgrade epizootiological area confirmed that 70.1% of horses have been infected with West Nile fever virus at least once in the past in their lifetimes, but we found 5.1% of horses were new cases from the last (2019) season as well. A trend of increasing West Nile virus seroprevalence in horses in the Belgrade area is evident. Results correlate with the occurrence of West Nile fever in the human population in Belgrade, in which West Nile fever has been present for the last few years including in summer 2019 (the time of this study). The horse population could serve as a sentinel for the overall prevalence of West Nile fever in a defined territory.

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## **Authors' contributions**

LjV have made substantial contributions to basic idea, conception and design, carried out analysis of data and interpretation of results and have been involved in drafting the manuscript. Autor LjV have given final approval of the version to be published as well.

SS and IP made contributions in the process of collection of specimens from horses, on the field and in preparation samples for further analysis as well. JVM and VR carried out serology analysis of presence of IgG in horses sera and data analysis. JZ and NJ carried out serology analysis of presence of IgM in horses sera and data analysis. BK and VM revised the manuscript critically and together with LjV prepared the final draft of the manuscript etc. All authors read and approved the final manuscript.

## **Competing interests**

The authors declare that they have no competing interests.

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## **SEROPREVALENCA GROZNICE ZAPADNOG NILA KOD KONJA NA EPIZOOTIOLOŠKOM PODRUČJU GRADA BEORADA**

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### **Kratak sadržaj**

Groznica zapadnog Nila je vektorska bolest virusne etiologije od koje mogu oboleti konji, ptice i ređe neke druge vrste životinja. Svake godine groznica zapadnog Nila je detektovana i u humanoj populaciji u Srbiji. Bolest se često ispoljava u subkliničkoj formi ali najčešće se kao klinički manifestna bolest javlja kod konja. Zbog toga su konji preporučeni kao sentinel vrsta za monitoring i praćenje opšte incidence groznice zapadnog Nila na određenoj teritoriji. Naš cilj je bio da proverimo prisustvo specifičnih antitela protiv groznice zapadnog Nila kod konja na epizootiološkom području grada Beograda. Ispitali smo imunološki status 77 konja i dobili informacije o prevalenci

groznice zapadnog Nila kod konja na teritoriji epizootiološkog područja grada Beograda. Serumi su testirani korišćenjem ELISA testa za detekciju specifičnih antitela IgG klase protiv groznice zapadnog Nila i ELISA testa za detekciju antitela IgM klase da bismo utvrdili postojanje starih i skorašnjih akutnih infekcija groznice zapadnog Nila kod konja. Rezultati ukazuju da je groznica zapadnog Nila rasprostranjena kod 70,1% konja u populaciji na teritoriji grada Beograda. Detektovali smo takodje i prisustvo akutne skorašnje infekcije groznice zapadnog Nila kod 5,1% konja u poslednjoj sezoni bolesti tokom 2019. godine. Evidentan je trend rasta seroprevalence groznice zapadnog Nila kod konja na teritoriji Beograda.

**Ključne reči:** IgG antitela, IgM antitela, ELISA test, Groznica zapadnog Nila, vektorske bolesti, Beograd